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Thomas P. Ahern

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14. ABSTRACT
In the first year of his predoctoral award, Thomas Ahern completed all coursework and qualifying examinations required for entry into the senior phase of doctoral study for the Doctor of Science degree in epidemiology at Boston University. Mr. Ahern submitted a dissertation research proposal to the Epidemiology Doctoral Committee in May 2008; the proposal was formally approved in October 2008. Mr. Ahern completed and published one of the three studies of his dissertation research in December 2008, an investigation of the effects of cardiac glycoside treatment on breast cancer risk. Mr. Ahern has supervised the ongoing collection and processing of biological samples required for his second and third dissertation projects. These studies are on track for completion in 2010. Meanwhile Mr. Ahern has published several other breast cancer research papers as a supplement to his training program.

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Introduction:

Thomas Ahern is a candidate for the Doctor of Science (D.Sc.) degree in epidemiology at the Boston University School of Public Health. Mr. Ahern's predoctoral training program includes advanced epidemiology and biostatistics coursework, collaboration on an international, multidisciplinary research team to study molecular and genetic determinants of breast cancer outcomes, teaching assistantships and guest lectures in epidemiologic methods courses, and a dissertation project focused on (1) the impact of prescription drugs on breast cancer risk, (2) the association of p21-activated kinase 1 (PAK1) overexpression with tamoxifen effectiveness, and (3) effects of functional polymorphisms in the UDP-glucuronosyltransferase (UGT) enzymes on breast cancer recurrence in Danish women treated with tamoxifen.

Body:

Thomas Ahern completed all required coursework for his doctoral degree in May of 2008. Just prior to this, he successfully passed both required sections of the doctoral qualifying examination for epidemiology (epidemiology section: Summer 2007; biostatistics section: Winter 2007). These achievements enabled Mr. Ahern to embark on his dissertation research.

Two of Mr. Ahern's three dissertation studies are nested within a population-based case-control study of breast cancer recurrence among Danish women (PI: Timothy L. Lash, Chair of Mr. Ahern's Doctoral Committee). In accordance with the Statement of Work, Mr. Ahern traveled to Aarhus University in the first year of his training program to oversee the commencement of tissue processing and DNA extraction operations in the collaborating laboratory. Mr. Ahern has also participated in investigator teleconferences to coordinate the activities of study collaborators and troubleshoot logistical and technical challenges.

After finalizing the roster of study participants, breast cancer diagnosis and treatment data were extracted from the Danish Breast Cancer Cooperative Group (DBCG) database. Additional data on the prescription drug history of cases and controls were extracted from county pharmacy databases. These data permitted Dr. Lash, Mr. Ahern and their collaborators to conduct a study of the effects of the CYP2D6-inhibiting antidepressant, citalopram, on the breast cancer recurrence rate among women with ER-positive resected breast tumors who were treated with tamoxifen.[1] Procurement of archived tumor tissue from all cases and controls—critical to Mr. Ahern's genotyping and protein expression studies—is better than 50 percent complete as of this writing. An unforeseen technical delay in the laboratory—beyond Mr. Ahern's control—required the replacement of one of Mr. Ahern's

originally planned studies. This change to the training program, reflected in a revised Statement of Work, was approved by CDMRP in February 2009. The substitute study examined the association between cardiac glycoside treatment (e.g., digoxin) and breast cancer incidence, using a population-based case-control design. This study was completed and published in 2008 (a copy of the manuscript appears in the appendix).[2]

Beyond the research aims outlined in the Statement of Work, Mr. Ahern conducted three additional breast cancer studies which have augmented his training program. The first of these studies characterized the temporal trend in breast-conserving surgery use by Danish surgical oncologists and their patients, in relation to the publication of three major trials demonstrating survival equivalency of the two procedures.[3] The second of these studies explored the effect of comorbid disease on all-cause mortality in breast cancer survivors, where comorbidity status was either assessed at breast cancer diagnosis or updated repeatedly throughout follow-up.[4] The third study explored the association between lifetime exposure to tobacco smoke and the incidence of breast cancer (the manuscript is presently under review). Copies of the manuscripts are attached in the appendix.

Key Program Accomplishments:

- Successfully passed required qualifying examination (epidemiology and biostatistics sections) for DSc degree in epidemiology, Fall 2007
- Completed required coursework for DSc degree in epidemiology, May 2008.
- First dissertation study (exploring association between digoxin treatment and breast cancer risk) conducted and published.[2]
- Co-authored an original research paper on the modification of tamoxifen's effectiveness by the CYP2D6-inhibiting antidepressant citalopram.[1]
- Co-authored two letters to journal editors with Dr. Lash and members of the Danish collaboration.[5, 6]
- Served as a teaching assistant for EP854, "Modern Epidemiology" (Professor Timothy Lash); Fall 2006 and Fall 2007.
- Served as a teaching assistant for EP755, "Infectious Disease Epidemiology" (Professors Barbara Mahon, Matthew Fox and Robert Horsburgh); Spring 2007 and Spring 2008.
- Served as a teaching assistant for EP712, "Epidemiologic Methods" (Professor Dan Brooks); Fall 2008.
- Delivered a guest lecture on the conduct of case-control studies to EP711, "Introduction to Epidemiology" (Professor Elizabeth Lawler and Ryan Ferguson); Fall 2008.

- Orally presented results from first dissertation study (digoxin and breast cancer) at the Epidemiology Department's Research in Progress seminar; Fall 2008.
- Gave a poster presentation of a study of acquired comorbidity and mortality among breast cancer patients at Boston University's Science and Engineering Research Symposium; Spring 2008.

Reportable Outcomes:

- Publication of four original breast cancer research papers, one of which applies toward the research requirements for the DSc degree in epidemiology.[1-4] Copies of these papers appear in the appendix of this report.

Conclusion:

Mr. Ahern has made substantial progress toward the completion of the D.Sc. degree in epidemiology in the first year of his CDMRP predoctoral award. He published the largest study to date of the effect of cardiac glycoside treatment on breast cancer risk,[2] in addition to other breast cancer studies beyond those that form his dissertation research.[1, 3, 4] These contributions to the breast cancer literature provide new knowledge to scientists and clinicians in the field, and will help to advance breast cancer risk assessment and treatment technology.

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Appendix:

The following pages contain copies of manuscripts authored or co-authored by Mr. Ahern in the first year of his predoctoral award, and an updated copy of his curriculum vitae.

Research article

Open Access

Digoxin treatment is associated with an increased incidence of breast cancer: a population-based case-control study

Thomas P Ahern[1,2], Timothy L Lash[1,2], Henrik T Sørensen[1,2] and Lars Pedersen[2]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, 8200 Aarhus N, Denmark
[2]Department of Epidemiology, Boston University School of Public Health, 715 Albany Street T3E, Boston, MA 02118, USA

Corresponding author: Thomas P Ahern, tpa@bu.edu

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Abstract

Introduction Laboratory and epidemiologic studies have suggested a modifying effect of cardiac glycosides (for example, digoxin and digitoxin) on cancer risk. We explored the association between digoxin treatment and invasive breast cancer incidence among postmenopausal Danish women.

Methods We used Danish registries to identify 5,565 postmenopausal women diagnosed with incident invasive breast carcinoma between 1 January 1991 and 31 December 2007, and 55,650 matched population controls. Cardiac glycoside prescriptions were ascertained from county prescription registries. All subjects had at least 2 years of recorded prescription drug and medical history data. We estimated the odds ratio associating digoxin use with breast cancer in conditional logistic regression models adjusted for age, county of residence, and use of anticoagulants, non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, and hormone replacement therapy. We also explored the impact of confounding by indication and detection bias.

Results Digoxin was the sole cardiac glycoside prescribed to subjects during the study period. There were 324 breast cancer cases (5.8%) and 2,546 controls (4.6%) with a history of digoxin use at least 1 year before their index date (adjusted odds ratio (OR): 1.30; 95% confidence interval: 1.14 to 1.48). The breast cancer OR increased modestly with increasing duration of digoxin exposure (adjusted OR for 7 to 18 years of digoxin use: 1.39; 95% confidence interval: 1.10 to 1.74). The association was robust to adjustment for age, receipt of hormone replacement therapy, coprescribed drugs, and confounding by indication. A comparison of screening mammography rates between cases and controls showed no evidence of detection bias.

Conclusions Our results suggest that digoxin treatment increases the risk of invasive breast cancer among postmenopausal women.

Introduction

Cardiac glycosides (CGs) are natural steroid toxins that have been used since the 18th century to treat congestive heart failure (CHF) and atrial fibrillation (AF) [1]. The clinically most prevalent CGs are the *Digitalis*-derived cardenolides digitoxin and digoxin. These compounds exert their pharmacologic effect via inhibition of the Na^+/K^+ ATPase, which indirectly raises intracellular Ca^{2+} concentration, thus increasing the force of contractility in cardiac myocytes.

In 1979, Stenkvist *et al.* reported an unusual finding in a small cohort of breast cancer patients (n = 142) [2]. Women in the cohort who were taking CGs (mostly digoxin) at the time of their breast cancer diagnosis had tumors with less aggressive phenotypes than breast tumors of women not taking CGs [2]. They later reported a higher recurrence rate among the women not taking CGs after 5 [3] and approximately 22 [4] years of follow-up. These observations suggested a beneficial effect of cardiac glycosides for women with breast tumors. An early mechanistic hypothesis centered on CG interference with estrogen receptor (ER) signaling in tumor cells [2], while current laboratory studies implicate novel signaling pathways mediated by the Na^+/K^+ ATPase [5,6].

AF: atrial fibrillation; BMI: body mass index; CG: cardiac glycoside; CHF: congestive heart failure; CI: confidence interval; CVD: cardiovascular disease; ER: estrogen receptor; HRT: hormone replacement therapy; ICD: International Classification of Diseases; OR: odds ratio; SIR: standardized incidence ratio.

Subsequent studies of the association between CG use and breast cancer incidence gave conflicting results. Haux *et al*. compared site-specific cancer incidence rates among digitalis-treated Norwegians patients with expected rates in the general population [7]. Several cancers, including female breast cancer, occurred at higher rates among those treated with digitalis compared with the general population [7]. Also, Friedman reported no association between CG prescription history and breast cancer in a Kaiser-Permanente registry study [8].

Given the continued importance of CG medicines to treat heart disease and the inconsistent results from earlier studies of the association between this therapy and breast cancer occurrence, we examined the association between digoxin treatment and breast tumor incidence rate in a population-based prospective case-control study of postmenopausal Danish women.

Materials and methods

This study was approved by the Boston University Medical Campus Institutional Review Board and the Danish Registry Board.

Study population

This study was conducted within the female population of North Jutland and Aarhus Counties, Denmark [9]. We used county hospital registries to ascertain all cases of incident invasive breast cancer diagnosed in women age 55 or older. Ascertainment began on 1 January 1991 in North Jutland County and 1 January 1998 in Aarhus County, and continued until 31 December 2007 [10]. The hospital registries contain data on patients' civil personal registry (CPR) number, date(s) of admission, date(s) of discharge, and up to 20 discharge diagnoses and medical procedures per discharge or outpatient visit. Diagnoses are assigned by the attending physician, and are coded according to the *International Classification of Diseases*, 8th revision (ICD-8, until 1995) and 10th revision (ICD-10, 1995 onwards).

Controls were identified in the Danish Civil Registration System, which has tracked residential address, vital status, and date of emigration for the entire Danish population since 1968 [11]. Controls were selected for each case by risk-set sampling, matching controls to cases on year of birth and county of residence. Within strata of the matching factors, we selected 10 controls at random among those who were alive and without a history of breast cancer on the date of the matched case's diagnosis. This date was the index date for the cases and matched controls.

Data collection

We used each subject's unique CPR number to link the case-control roster to county prescription databases [12,13], which automatically record all prescriptions filled since 1989 in North Jutland County and 1996 in Aarhus County. The databases encode drugs by the Anatomical Therapeutic Chemical (ATC) classification system [14] and record dates of all prescription fills along with the patient's CPR number. These systems report prescription data to the county databases, as well as to the Danish National Health Service, which refunds a portion of medication costs. Prescriptions are logged in the registries after patients present to a pharmacy and pay their share of the prescription cost. To ensure adequate prescription data history, we excluded cases and controls who had lived in the study counties for less than 2 years after the establishment of electronic prescription registries. We ascertained medical history for cases and controls by extracting major diagnoses preceding index dates from the county hospital registries. We also used these registries to identify all prediagnosis mammography procedures for cases and controls since 2001, the year mammography data began to be systematically recorded.

Definitions of analytic variables

We identified cases of incident breast cancer in the hospital registries using ICD-8 and ICD-10 codes appropriate to the date ranges of the databases. ICD codes were also used to ascertain comorbid conditions for cases and controls (see full ICD code listing in Table 1).

We ascertained CG prescriptions by extracting all records from the prescription databases with ATC codes beginning with C01A. CGs are available only by prescription in Denmark, and are dispensed at pharmacies equipped with automated electronic reporting systems described in the data collection section. This strategy captured all CG prescriptions in the counties over the study period that were for digoxin exclusively. Digoxin prescriptions were only considered if they occurred at least 1 year before the index date. Digoxin exposure was considered in broad terms as ever exposed (≥ 1 digoxin prescription at least 1 year before the index date) or never exposed (no record of digoxin prescription at least 1 year before the index date), and in finer terms according to the length of time between a woman's first digoxin prescription and her index date.

Confounders were selected *a priori* based upon established breast cancer risk factors that were also likely to influence receipt of digoxin. Age was initially controlled by matching cases to controls on year of birth. We also calculated each subject's exact age on her index date to adjust for residual confounding by age. We additionally considered confounding by coprescription of anticoagulants, non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, and hormone replacement therapy (HRT). Anticoagulants are frequently prescribed for AF, and were associated with lower risk of urogenital cancer [15]. NSAID use has been associated with increased risk of CHF [16], and these drugs have shown protective associations with breast cancer in some studies [17]. Aspirin use, which may be more prevalent among digoxin users, has been

Table 1

Listing of ICD-8 and ICD-10 codes used to ascertain key diagnoses

Diagnosis	ICD-8	ICD-10
Invasive breast carcinoma[a]	174.00 to 174.02; 174.08; 174.09;	C50.0 to C50.6; C50.8; C50.9
Congestive heart failure	427.09; 427.10; 427.11; 427.19; 428.99; 782.49	I50; I11.0; I13.0; I13.2
Atrial fibrillation/flutter[b]	427.93; 427.94	I48
Myocardial infarction	410	I21; I22; I23
Peripheral vascular disease	440; 441; 442; 443; 444; 445	I70; I71; I72; I73; I74; I77
Cerebrovascular disease	430 to 438	I60 to I69; G45; G46
Chronic pulmonary disease	490 to 493; 515 to 518	J40 to J47; J60 to J67; J68.4; J70.1; J70.3; J84.1; J92.0; J96.1; J98.2; J98.3
Mild liver disease	571; 573.01; 573.04	B18; K70.0 to K70.3; K70.9; K71; K73; K74; K76.0
Moderate to severe liver disease	070.00; 070.02; 070.04; 070.06; 070.08; 573.00; 456.00 to 456.09	B15.0; B16.0; B16.2; B19.0; K70.4; K72; K76.6; I85
Diabetes type 1	249.00; 249.06; 249.07; 249.09	E10.0, E10.1; E10.9
Diabetes type 2	250.00; 250.06; 250.07; 250.09	E11.0; E11.1; E11.9
Moderate to severe renal disease	403; 404; 580 to 583; 584; 590.09; 593.19; 753.10 to 753.19; 792	I12; I13; N00 to N05; N07; N11; N14; N17 to N19; Q61
Diabetes with end organ damage (types 1 and 2)	249.01 to 249.05; 249.08; 250.01 to 250.05; 250.08	E10.2 to E10.8; E11.2 to E11.8
Solid tumor	140 to 194	C00 to C75
Lymphoma	200 to 203; 275.59	C81 to C85; C88; C90; C96

[a]The ICD codes for invasive breast carcinoma do not capture *in situ* tumors (for example, intraductal carcinoma); [b]ICD-8 contained separate codes for atrial fibrillation (427.93) and flutter (427.94). These two diagnoses were combined into a single code in ICD-10 (I48).
ICD, International Classification of Disease.

associated with reduced breast cancer risk [18], though data are conflicting [17]. We therefore evaluated confounding by low- and high-dose aspirin use. We also evaluated HRT as a confounder because of its contribution to cumulative hormonal exposure and its association with breast cancer risk [19].

Prescriptions for hormone replacement therapy were identified by ATC codes (estrogens: codes starting with either G03C or L02AA; progestin: codes beginning with G03D; combination therapy: codes beginning with either G03F or G03H). Exposure to any of these drugs before the index date was classified as 'ever exposed to HRT' while exposure to none of them was classified as 'never exposed to HRT'. Similarly, we characterized ever/never exposure to anticoagulants, NSAIDs and aspirin by searching for ATC codes beginning with B01A, M01A, and B01AC06, respectively.

We evaluated confounding by the medical indications for digoxin therapy by defining an alternative reference group of women who were never exposed to digoxin and who had a history of cardiovascular disease (excluding CHF or AF). We hypothesized these reference subjects should be more similar to the digoxin-treated women with regard to cumulative hormonal exposures and lifestyle factors that may modify risk for both heart disease and breast cancer. This reference group also facilitated evaluation of detection bias by allowing comparison of digoxin-exposed women to women with other serious histories who would likely have similar medical usage patterns. We further evaluated detection bias by comparing mammography usage rates between cases and controls. Dates of all mammography procedures among cases and controls were identified in hospital registries using appropriate Danish medical procedure codes. We analyzed mammography usage among women with index dates from 1 January 2006 onward, the period of our study when screening mammography would have been most common in Denmark. For each subject who had undergone mammography before her index date, we identified her most recent procedure and calculated the time elapsed between that procedure and the index date.

Statistical analysis

We characterized the names, doses, and prescribing frequencies of the various digoxin products used over the study period. We computed the frequency and proportion of cases and controls by digoxin exposure status, prevalent medical

conditions, use of other prescription drugs (HRT, anticoagulants, NSAIDs and aspirin), and age on index date.

We calculated the unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) associating digoxin exposure categories with incident breast cancer and used conditional logistic regression to account for the matching factors and to adjust for exact age and past use of HRT, anticoagulants, NSAIDs, and aspirin. Due to the risk-set sampling design, the odds ratio approximates the incidence rate ratio associating digoxin exposure with incident breast cancer [20]. All analyses were performed with SAS version 9 (SAS Institute, Cary, NC, USA).

Results

Characteristics of cases and controls

We identified 5,565 cases and 55,650 matched population controls. Among the cases, 324 (5.8%) had ever had a digoxin prescription at least 1 year before her diagnosis date and 2,546 (4.6%) of controls had ever had a digoxin prescription at least 1 year before her index date. The distributions of cases and controls according to age, mammography usage, comorbidity and relevant prescription drug usage are shown in Table 2. By virtue of the matching, cases and controls were identical with respect to age distribution. Cases were somewhat more likely to have CHF, AF, chronic pulmonary disease, or diabetes, and were less likely to have a history of myocardial infarction, than controls. Cases also had more exposure to HRT, anticoagulants and NSAIDs than controls. As expected, mammography usage was substantially higher for cases than for controls in the year preceding the index date (81% vs 1.6%, respectively). However, usage was similar for cases and controls in time periods more distant from index dates.

Digoxin treatment and incident breast cancer

Table 3 shows all of the cardiac glycoside products recorded in the county prescription registries during the study period. We noted that digoxin was the sole CG used during this period. Approximately 97% of all digoxin prescriptions were for 62.5 μg tablets, indicating very little product heterogeneity among the digoxin-exposed subjects.

We observed a higher rate of breast cancer among ever-users of digoxin, relative to never users, in both unadjusted and adjusted analyses (adjusted OR: 1.30; 95% CI: 1.14 to 1.48; Table 4). This association persisted in categories of drug exposure duration (1 to 3 years, 4 to 6 years and 7 to 18 years), with a suggested upward trend in the odds ratios with increasing duration of digoxin therapy. When we compared digoxin-exposed women with the alternative reference group of unexposed women with cardiovascular medical histories, we continued to observe an association between digoxin exposure and incident breast cancer (adjusted OR: 1.42; 95% CI: 1.14 to 1.77).

Discussion

Our results suggest there may be a causal association between digoxin treatment and incident breast cancer in postmenopausal women. These findings were robust to adjustment for key confounders, confounding by indication, and medical detection bias.

Interestingly, results from a case-control study by Stenkvist *et al*. agree with our present findings. The investigators compared the CG exposure history of the breast cancer cases from their original report [2] to the exposure history of age-matched controls from the general population [21]. The authors concluded that CGs had no influence on breast cancer incidence, due to a non-significant chi-squared test for independence ($p = 0.25$). The data from the published cross-tabulation in fact yield an OR of 1.39, with a 95% CI of 0.79 to 2.45. While the interval is somewhat wide, the OR is near to our result and consistent with a causal association between CG use and incident breast cancer.

Other previous research is consistent with our results [7,22]. Haux and colleagues observed an elevated breast cancer rate (standardized incidence ratio (SIR): 1.25; 95% CI: 0.95 to 1.62) among mostly postmenopausal digitoxin users, compared with the rate in the general population [7]. The authors also observed elevated SIR for several other cancer sites [7]. Friedman reported results from a Kaiser Permanente cohort study of carcinogenic effects of prescription drugs, which showed no statistically significant association between digitalis treatment and breast cancer incidence. However, the SIR for this association was 1.2 – similar to the result of our study. Ewertz *et al*. found a positive association between digoxin usage and incident male breast cancer (OR for = 5 years of digoxin use: 2.0; 95% CI: 0.9 to 4.4) [22]. Together these results argue against ER antagonism by digitalis glycosides. Our results are more consistent with an ER agonist property of digoxin, though some *in vitro* ER binding studies do not support this notion [23,24].

Recent laboratory findings implicate the Na^+/K^+ ATPase in a variety of signal transduction pathways, with end effects in cell adhesion, survival, and proliferation [25]. Several *in vitro* studies point toward a downstream antiproliferative effect of CGs but others leave open the possibility of cancer-promoting endpoints [26]. The interaction of cardiac glycosides with the Na^+/K^+ ATPase and the consequential effects appear to be highly dependent on the specific CG compound and the subunit makeup of the receiving ATPase [6,26,27]. Therefore it would not be surprising to observe inconsistent responses of different human tissue types to the diverse cardiac glycosides. Some of these ligand-, receptor-, and tissue-specific responses may plausibly result in breast tumorigenesis *in vivo*, consistent with our findings. With this study, we have isolated the association between a single cardiac glycoside, digoxin,

Table 2

Characteristics of the study sample

Variable	Cases (n = 5,565)	Controls (n = 55,650)
Age on index date (years):		
55 to 64	2,116 (38)	21,160 (38)
65 to 74	1,800 (32)	18,000 (32)
75 to 84	1,356 (24)	13,560 (24)
≥ 85	293 (5.3)	2,930 (5.3)
Medical history, n (%):		
Congestive heart failure	160 (2.9)	1,337 (2.4)
Atrial fibrillation/flutter	224 (4.0)	1,819 (3.3)
Prediagnosis mammography[a]		
< 1 year:	417 (81)	84 (1.6)
1 to < 2 years:	3 (0.6)	84 (1.6)
2 to < 3 years:	9 (1.7)	84 (1.6)
≥ 3 years:	17 (3.3)	130 (2.5)
Myocardial infarction	123 (2.2)	1,492 (2.7)
Chronic pulmonary disease	334 (6.0)	3,125 (5.6)
Peripheral vascular disease	167 (3.0)	1,563 (2.8)
Cerebrovascular disease	275 (4.9)	2,842 (5.1)
Lymphoma	12 (0.2)	155 (0.3)
Other solid tumor	0	0
Liver disease	44 (0.8)	403 (0.7)
Diabetes (type I or II)	215 (3.9)	1,706 (3.1)
Diabetes with end-organ complication	85 (1.5)	591 (1.1)
Renal disease	35 (0.6)	446 (0.8)
Other drug exposures, n (%):		
Hormone replacement therapy	2,062 (37)	17,582 (32)
Anticoagulants	231 (4.2)	2,109 (3.8)
NSAIDs	3,106 (56)	29,964 (54)
Aspirin, low-dose (< 150 mg)	205 (3.7)	2,004 (3.6)
Aspirin, high-dose (≥ 150 mg)	505 (9.1)	4,878 (8.8)

[a]Screening mammography data were only available from 2001 onwards. We restricted the mammography analysis to cases and controls with index dates after 1 January 2006, when screening mammography would have been most common in Denmark. Categories reflect time elapsed between most recent mammogram and index date; proportion denominators are the total number of cases (n = 516) or controls (n = 5,160) in the restricted data set.
NSAID, non-steroidal anti-inflammatory drug.

Table 3

All cardiac glycoside products prescribed to study subjects[a]

Product name	Dose	Fill quantity	No. of prescriptions, (% of total)
Digoxin	62.5 μg/tablet	100 tablets	83,094 (66)
	62.5 μg/tablet	200 tablets	38,188 (31)
	250 μg/tablet	100 tablets	4,047 (3.2)
	50 μg/mL	30 mL	28 (0.02)

[a]Result of searching the prescription database for all Anatomical Therapeutic Chemical (ATC) codes beginning with 'C01A'.

and breast cancer incidence in a virtually unselected population of postmenopausal women.

Strengths and limitations

The main strengths of this study are its large size, use of high-validity registry data to ascertain diagnoses, use of prospectively-recorded exposure information, and lack of selection in enumerating cases and controls.

Our study design minimized the threat of selection bias, which can create the illusion of an exposure-disease association when, in fact, none exists [28]. We had only one subject exclusion criterion, and controls were selected completely at random within strata of the matching factors. Since no subject was required to give their consent to participate, no self-selection mechanism could have influenced our results.

Our results are subject to distortion by residual confounding and misclassification of exposure and outcome. We took measures to address confounding by age, past exposure to other prescription drugs, and the medical indications for digoxin prescription. We saw little change in the unadjusted association after accounting for these factors. Digoxin is ordinarily prescribed at an age when most women no longer bear children, so it is unlikely that digoxin exposure is strongly associated with the well-characterized reproductive factors that affect breast cancer risk [29]. We therefore do not expect substantial residual confounding. It is unlikely that use of other prescription drugs could bias our results, since antibiotics,

Table 4

Associations between digoxin treatment and incident breast cancer

Exposure categories	Cases (n = 5,565)	Controls (n = 55,650)	Unadjusted OR (95% CI)	Adjusted[a] OR (95% CI)
Ever/never prescribed digoxin:				
Ever user	324	2,546	1.29 (1.14 to 1.45)	1.30 (1.14 to 1.48)
Never user	5,241	53,104	1.0 (Ref)	1.0 (Ref)
Duration of digoxin therapy:[b]				
7 to 18 years	93	694	1.35 (1.10 to 1.69)	1.39 (1.10 to 1.74)
4 to 6 years	103	811	1.29 (1.05 to 1.58)	1.30 (1.05 to 1.61)
1 to 3 years	128	1,041	1.25 (1.03 to 1.50)	1.25 (1.03 to 1.52)
Never user	5,241	53,104	1.0 (Ref)	1.0 (Ref)
Ever/never prescribed digoxin (alternate reference group):	(n = 732)	(n = 7,086)		
Ever user	324	2,546	1.42 (1.21 to 1.65)	1.42 (1.14 to 1.77)
Never user[c]	408	4,540	1.0 (Ref)	1.0 (Ref)

[a]Adjusted for age (continuous), county of residence (categorical), and past receipt of hormone replacement therapy, anticoagulants, high- and low-dose aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) (ever/never); [b]years elapsed between first digoxin prescription and index date (approximate tertiles of the distribution); [c]the alternate reference group is additionally defined by a history of myocardial infarction, peripheral vascular disease, cerebrovascular disease, or any combination thereof. See text for rationale.
CI, confidence interval; OR, odds ratio.

antihypertensives, statins, and antidepressants do not appear to modify breast cancer risk [17]. Use of the alternative reference group resulted in a modest increase in the estimated odds ratio; this result implies that confounding by indication actually served to attenuate the original association. Furthermore, detection bias is not likely to account for the observed association, since women with other cardiovascular diseases would have similar medical usage to women treated with CG. In the whole study population, we saw no material difference in mammography usage rates between cases and controls in time periods distant from index dates, which further argues against detection bias.

We were not able to adjust directly for body mass index (BMI), which is associated with both cardiovascular disease (CVD) and breast cancer [30]. However our alternative reference group likely controlled in part for BMI due to the association of BMI with CVD [31]. Since the effect of adjustment via this reference group was to move the odds ratio estimate away from the null, it is unlikely that unmeasured confounding by BMI could account for our positive result.

Our characterization of digoxin exposure was informed only by the number and strength of prescriptions filled by study participants; the prescription registry data did not permit calculation of actual daily doses taken by exposed subjects. Because prescription records were generated automatically before breast cancer diagnoses, we expect any exposure classification error to be non-differential in nature. We are not aware of published validation data on the classification of incident breast cancer in the hospital discharge registries. However, breast cancer diagnoses were recorded without express knowledge of exposure, so outcome misclassification is also expected to be non-differential. Since non-differential classification errors are expected to attenuate results, exposure and outcome misclassification cannot plausibly account for our positive association [28].

Conclusion

We observed a modestly increased rate of breast cancer among postmenopausal women with any history of digoxin use, compared with women with no such use, after adjustment for age, use of other prescription drugs, and cardiovascular indications. The associations persisted in long-term exposure categories. While a number of laboratory studies of cardiac glycosides and female breast cancer have suggested protective effects, our results suggest that one specific cardiac glycoside, digoxin, moderately increases the incidence rate of breast cancer. This finding agrees with results from past studies; [7,8,21] the importance of which were likely masked by large standard errors of the association measures.

Competing interests

HTS reports receiving no fees, honoraria, grants or consultancies. The Department of Clinical Epidemiology is, however, involved in studies with funding from various pharmaceutical companies (Amgen, Pfizer, Glaxo and Centocor) as research grants to (and administered by) Aarhus University. None of these studies have relation to the present study. TPA, LAP and TLL declare no conflicts of interest.

Authors' contributions

HTS, TPA and TLL conceived the study idea. TPA, TLL and HTS designed the study. HTS and LAP collected the data. TPA performed all data analyses, reviewed the literature and wrote the first draft of the manuscript. All authors edited the manuscript.

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Impact of Acquired Comorbidities on All-Cause Mortality Rates Among Older Breast Cancer Survivors

Thomas P. Ahern, MPH, Timothy L. Lash, DSc,*† Soe Soe Thwin, PhD,† and Rebecca A. Silliman, MD*†*

Background: Breast cancer survivors with higher numbers of comorbidities at the time of primary treatment suffer higher rates of all-cause mortality than comparatively healthier survivors. The effect of time-varying comorbidity status on mortality in breast cancer survivors, however, has not been well investigated.

Objective: We examined longitudinal comorbidity in a cohort of women treated for primary breast cancer to determine whether accounting for comorbidities acquired after baseline assessment influenced the hazard ratio of all-cause mortality compared with an analysis using only baseline comorbidity.

Methods: Cox proportional hazards adjusted for age, race/ethnicity, and exercise habits were modeled using (1) only a baseline Charlson index; (2) 4 Charlson index values collected longitudinally and entered as time-varying covariates, with missing values addressed by carrying forward the prior observation; and (3) the 4 longitudinal Charlson scores entered as time-varying covariates, with missing values multiply imputed.

Results: The 3 modeling strategies yielded similar results; Model 1 HR: 1.4 per unit increase in Charlson index, 95% confidence interval (CI): 1.2–1.7; Model 2 HR: 1.3, 95% CI: 1.1–1.5; and Model 3 HR: 1.4, 95% CI: 1.2–1.6.

Conclusions: Our findings indicate that a unit increase in the Charlson comorbidity index raises the hazard rate for all-cause mortality by approximately 1.4-fold in older women treated for primary breast cancer. The conclusion is essentially the same whether accounting only for baseline comorbidity or accounting for acquired comorbidity over a median follow-up period of 85 months.

Key Words: aged, chronic disease, breast neoplasms, comorbidity, mortality

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Breast cancer is primarily a disease of older women, who frequently have other diseases as well.[1,2] When present, these diseases may affect breast cancer treatment choices and adherence to treatment regimens,[2–8] which would directly affect breast cancer mortality and therefore affect overall mortality. Medical attention focused on the treatment of breast cancer may also detract from definitive care of comorbid disease, and therefore increase all-cause mortality rates in breast cancer patients. Evidence for this phenomenon has been reported for serious diseases other than breast cancer.[9,10]

Recent years have witnessed a surge of investigations into the role comorbidity plays in the treatment and care of older cancer patients. Past studies have examined the effect of comorbid conditions on cause-specific and all-cause mortality rates, showing that older breast cancer survivors with a greater burden of comorbidity suffer from higher rates of all-cause mortality than those who are healthier.[4,6,8,11,12] To date, no study of this association has accounted for changes in comorbidity beyond the period of initial cancer treatment. We have expanded upon previous research by accounting for acquired comorbidity and examining its effect on all-cause mortality in a cohort of older women diagnosed with early stage breast cancer.

METHODS

Study Population

We conducted our study within an ongoing prospective cohort of older women diagnosed with early stage breast cancer. The enrollment criteria and data collection procedures for this cohort have been described in detail elsewhere.[13] Briefly, women aged 65 years or older diagnosed with early stage breast cancer (stage I with tumor diameter ≥1 cm, stage II, or stage IIIa) between 1996 and 1999 at 1 of 61 hospitals in Rhode Island, North Carolina, Minnesota, or Los Angeles, were identified through tumor registries and hospital pathology reports. Women whose physicians gave contact permission were invited to participate in the study (n = 1621**)**. Additional entry criteria included the following: (1) no prior history of primary breast cancer, (2) no simultaneously diagnosed primary tumor at another anatomic site, (3) English-speaking or with an available translator, and (4) competent for interview with satisfactory hearing or with an available proxy respondent. Women who were not enrolled within 5 months of the date of their breast cancer surgery were excluded. Of the 1621 women whose physicians gave contact permission, 865 consented to participate in the study and were subsequently enrolled. All participants returned a signed consent form approved by local institutional review boards.

From the Departments of *Epidemiology, Boston University School of Public Health; and †Medicine, Boston University School of Medicine, Boston, Massachusetts.

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Reprints: Thomas P. Ahern, Boston University School of Public Health, 715 Albany Street, T3E, Boston, MA 02118. E-mail: tpa@bu.edu.

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Participants were interviewed by telephone at 3, 6, and 15 months, and annually thereafter until 87 months, after primary tumor treatment. These interviews collected data on patient demographics, lifestyle, primary tumor and treatment characteristics, cancer recurrence, and comorbid conditions.

Definition of Analytic Variables

The 3-month interview served as the baseline time point for all subjects, and we restricted the sample to those who successfully completed a baseline interview (n = 689). We calculated the number of person-days of follow-up for each individual by extracting the number of days between the date of baseline interview and either the date of death or the date of last completed interview. Of the 689 subjects, 4.1% did not have recorded interview dates but had indicator variables for having completed an interview at each follow-up month. For these subjects, person-days were estimated by multiplying the number of months between surgery and last follow-up by 30.5 days. Eighty-seven subjects were lost to follow-up between the baseline and month 75 interviews; a further 203 were lost to follow-up after the month 75 interview.

Age at the time of primary treatment was divided into 3 categories for descriptive purposes (65–69, 70–79, and >79 years), but was modeled as a continuous variable. Race/ethnicity was self-reported as white, African American, Hispanic, Asian/Pacific Islander, Native American or Other. Regular exercise was defined in the interview question as "physical activity for at least one-half hour a day at least 3 times per week, with physical fitness being the main purpose of the activity," exclusive of any exercises prescribed by a subject's physician or physical therapist. We asked about exercise habits at the 6, 15, 27, 39, 51, and 87 month interviews.

We collected comorbidity data from participants at the 3, 27, 51, and 75 month interviews. We calculated the Charlson index of comorbidity,[14] using a method adapted to interview data instead of medical record abstractions.[15] Briefly, we constructed a Charlson score for each subject at each time point by assigning specified weights to 15 contributing health conditions if present at the time of interview. We translated the sum of the accrued weights into the ordinal Charlson index, which ranges from 0 (no comorbidity) to 3 (serious comorbidity). Once a subject reported a health condition it was assumed to persist for the remainder of a subject's follow-up time. Therefore, a given subject's Charlson index could either remain static or increase, but could not decrease over their follow-up time. A description of the ordinal Charlson index is given in Table 1.

The outcome for our study was death from any cause, ascertained by vital status queries of the National Death Index (NDI), the Social Security Administration (SSA), the death index of the Centers for Medicare and Medicaid Services (CMS), or by proxy interview response. We ascertained cause of death through regular queries of the NDI.

Selection of Candidate Confounders

We used a directed acyclic graph (DAG) to identify a sufficient set of confounders for analytic control. A DAG encodes hypothesized relations between variables, which can aid in identifying confounders of a given exposure-disease association. Confounders in a DAG are variables along a causal path with arrows pointing to both the exposure and disease (see Greenland et al[16] for a more complete definition). Figure 1 depicts the hypothesized relationships among the variables that influence comorbidity and all-cause mortality. Using the backdoor test described by Greenland et al,[16] control for age, exercise habits, and race/ethnicity were minimally sufficient to address confounding of the association between comorbidity and all-cause mortality, presuming the causal diagram faithfully depicts the causal relations among the variables. In our causal graph, tumor and treatment characteristics appear on the causal path-

TABLE 1. Formation of the Charlson Index

Charlson Weight	Comorbid Conditions
0	No comorbid conditions
1	Heart attack or treated for heart failure Surgical treatment for peripheral vascular disease Stroke, blood clot, or transient ischemic attack without loss of limb function Asthma treated with medications Peptic ulcer disease diagnosed by endoscopy or barium swallow Diabetes treated with oral medication or insulin injections Rheumatoid arthritis treated with medications, lupus, or polymyalgia rheumatica Alzheimer disease or other dementia
2	Stroke, blood clot, or transient ischemic attack with reduced arm or leg function Poor kidney function, high blood creatinine, ever used hemodialysis or peritoneal dialysis, or kidney transplant Diabetes with end-organ complications Diagnosed leukemia, lymphoma, or polycythemia vera
3	Cirrhosis or serious liver damage

Prevalent comorbid conditions for each subject were assigned weights according to the table. The sum of the weights was then used to form the ordinal Charlson index (Charlson index of 0, no comorbid conditions; Charlson index of 1, sum of weights equal to 1 or 2; Charlson index of 2, sum of weights equal to 3 or 4; Charlson index of 3, sum of weights >5).

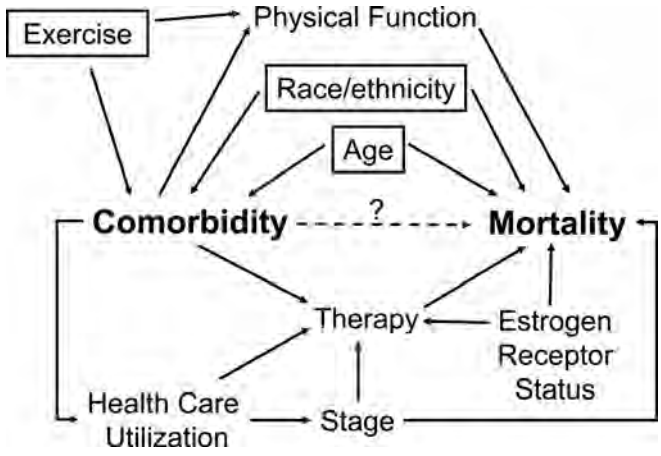

FIGURE 1. Directed acyclic graph depicting hypothesized relationships among covariates. Boxes indicate variables identified by backdoor test as confounders requiring adjustment. "Therapy" and "Stage" denote breast cancer treatment choices and AJCC disease staging, respectively.

way between comorbidity and all-cause mortality, making their control inappropriate. To do so would attenuate a portion of the total effect of comorbidity on all-cause mortality, leading to a biased measure of association.

Multiple Imputation of Missing Data

By design, all subjects had a baseline Charlson index. A considerable fraction of subjects (33%) had one or more missing values among their postbaseline Charlson index values, and 76% of subjects were missing one or more values among the 6 longitudinal exercise variables. To assess and correct for this loss to follow-up, we used a multiple imputation procedure to populate the missing data fields. A qualitative analysis of the data revealed nonmonotone patterns of missing values. That is, a missing value for either the Charlson index or exercise status at 1 time point did not always portend missing values at all future time points. Because of this nonmonotonicity, we were limited to using the Markov Chain Monte-Carlo (MCMC) imputation method. The MCMC method imputes continuous values for missing observations by drawing a specified number of fair random samples from a distribution characterized by the known values. Multiple imputation yields estimates of association that incorporate uncertainty about the imputed values into the variance of a parameter estimate, thus widening confidence intervals.[17] Five imputations were performed for each subject, using a single Markov chain and a noninformative prior distribution for the means and covariances of the missing Charlson and exercise data. Because the Charlson index is an ordinal measure, we constrained the range of imputed values between 0 and 3 and rounded to the nearest integer. The imputed dichotomous exercise variables were treated in an analogous manner.

To evaluate the performance of the multiple imputation procedure we selected a random sample of 20 subjects from those with complete Charlson data over the follow-up period (n = 462), recoded the subset's Charlson index values as missing, and imputed these values as described above. We compared the 5 imputed values at each follow-up point with the corresponding observed values and found that, overall, the imputed values matched the observed values 67% of the time. If the observed Charlson value was zero, the imputation matched 73% of the time, compared with a 56% match rate if the observed value was not equal to zero.

Statistical Analysis

We tabulated the number of subjects, cases of death, and person-days for the entire cohort based on sociodemographic, therapeutic, and comorbidity characteristics (Table 2). We modeled Cox proportional hazards to examine the effect of comorbidity on all-cause mortality when: (1) only baseline Charlson index was modeled; (2) the Charlson index was entered as a time-varying covariate, with missing values in the longitudinal scores addressed by carrying forward the last known observation; and (3) Charlson index was entered as a time-varying covariate, using imputed scores in place of the missing values. For the last of these procedures, 5 separate Cox models were obtained, one for each of the 5 imputations, and the results were combined to yield a single parameter estimate and standard error, accounting for the within- and between-imputation variability.[18] All models were additionally adjusted for age (continuous), race/ethnicity (nonwhite vs. white), and time-varying exercise habits (coded as a yes or no response to the regular exercise interview question). Models 1 and 2 were restricted to subjects who had nonmissing baseline exercise status (n = 612), and missing values for longitudinal exercise habits were

TABLE 2. Observed Baseline Characteristics of the Cohort (n = 689)

	No. Deaths	No. Subjects	Total Person-Days (%)
Age (yrs)			
65–69	33	176	395,769 (28)
70–79	112	383	822,089 (57)
>79	73	130	215,425 (15)
Race/ethnicity			
White	197	643	1,353,934 (94)
African American	15	34	58,903 (4.1)
Hispanic	1	3	5748 (0.4)
Asian/Pacific Islander	0	1	2669 (0.2)
Native American	1	1	916 (0.1)
Other	4	7	11,112 (0.8)
Baseline Charlson index			
0	93	390	850,230 (59)
1	95	248	485,639 (34)
2	21	38	77,758 (5.4)
3	9	13	19,586 (1.4)
Baseline exercise status			
Exercises regularly	83	350	767,999 (59)
Does not exercise regularly	96	262	542,221 (41)
Enrollment site			
Los Angeles	45	152	331,732 (23)
Rhode Island	64	174	355,070 (25)
Minnesota	65	190	384,647 (27)
North Carolina	44	173	361,834 (25)
AJCC* stage			
I	95	351	749,254 (52)
IIA	64	207	441,773 (31)
IIB	41	103	193,505 (14)
IIIA	17	27	48,101 (3.4)
Surgical treatment			
BCS, plus radiation therapy	46	218	496,136 (35)
BCS, no radiation therapy	42	111	220,916 (16)
Mastectomy	119	330	665,189 (47)
Other	9	19	31,292 (2.2)
Estrogen receptor status			
Positive	152	510	1,072,027 (76)
Negative	38	93	184,500 (13)
Unknown	25	76	152,627 (11)
Tamoxifen prescription			
Ever prescribed	135	453	959,096 (67)
Never prescribed	71	203	417,637 (29)
Unknown	12	33	56,550 (3.9)

*American Joint Committee on Cancer staging system.

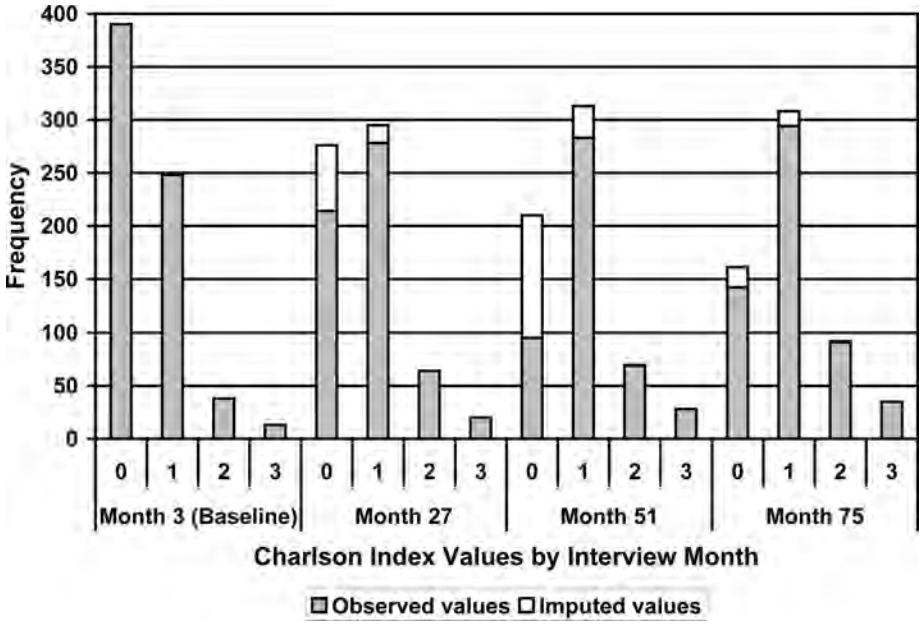

FIGURE 2. Distribution of observed and imputed Charlson index values by interview month following primary breast cancer surgery.

addressed by carrying forward the last known value. Imputed exercise status was used in Model 3.

The proportional hazards assumption for the Charlson index was verified for all 3 models by including a term for the interaction between Charlson index and the logarithm of person-days. The multiple imputation and statistical analyses were performed using SAS version 9 (SAS Institute, Cary, NC).

RESULTS

Six hundred eighty-nine subjects met the eligibility criteria and were included in the analysis. The total follow-up time was 3927 person-years, with a median individual follow-up time of 85 months. Table 2 displays the baseline characteristics of the analytic cohort. The majority of women in our study were white (95%), with a median age at enrollment of 73 years (range: 65–96 years). Most women (93%) began the study with a Charlson index of either 0 or 1, and 59% exercised regularly at baseline. Almost all of the participants (97%) had stage I or II breast cancer at diagnosis, and about half were treated with mastectomy. Of those who opted for breast-conserving surgery, only 66% received radiation therapy (RT).

Figure 2 shows the distribution of longitudinal Charlson index values before and after multiple imputation. Nearly all imputed Charlson index values were either 0 or 1, with little change in the proportion of moderate to severe comorbidity when combined with the measured values. The combined measured and imputed values demonstrate a trend toward more comorbidity over time, as expected.

Compared with the observed exercise values, the imputed values consistently showed a higher proportion of regular exercisers at each time point, but both sets showed an overall downward trend in the proportion of regular exercisers over time (data not shown).

Results from the 3 Cox models are shown in Table 3. Time-interaction terms for the Charlson index were nonsignificant in each of the 3 models ($P > 0.3$), thus verifying proportionality of hazards. The time-interaction terms were excluded from the final models. Each model result shows the relative increase in the hazard rate of death from any cause over our study's follow-up period (median: 85 months) associated with a 1-unit increase in the Charlson index. Model 1 considered only the baseline Charlson index while adjusting for age, race/ethnicity, and longitudinal exercise habits, with missing exercise values replaced by the last observation (HR: 1.4, 95% confidence interval [CI]: 1.2–1.7). Model 2 entered the Charlson index as a time-varying covariate, with missing

TABLE 3. Cox Proportional Hazards Models of All-Cause Mortality as a Function of Baseline or Acquired Comorbidity

Model	Parameter	Hazard Ratio*	95% CI	*P*
(1) Baseline Charlson	Charlson index	1.4	1.2–1.7	0.0004
	Age	2.4	1.9–3.0	<0.0001
	Race/ethnicity	1.6	1.0–2.7	0.06
	Exercise	1.1	0.9–1.3	0.19
(2) Time-varying Charlson index; missing values replaced by last known value	Charlson index	1.3	1.1–1.5	0.003
	Age	2.4	1.9–3.0	<0.0001
	Race/ethnicity	1.4	1.0–2.9	0.04
	Exercise	1.1	1.0–1.4	0.14
(3) Time-varying Charlson index; missing values multiply imputed†	Charlson index	1.4	1.2–1.6	0.0003
	Age	2.3	1.9–2.8	<0.0001
	Race/ethnicity	1.8	1.1–2.8	0.02
	Exercise	1.2	0.9–1.6	0.45

*Comparisons for hazard ratios; Charlson index, 1-unit increase in ordinal value; Age, 10-year increase; Race/ethnicity, nonwhite vs. white; Exercise, regular exercisers vs. nonregular exercisers.

†Missing values for exercise habits were also multiply imputed. Hazard ratio estimates and 95% confidence intervals were pooled over 5 imputations. *P* values are conservatively reported as the highest from among the 5 imputation models.

values replaced by the last known observation, adjusting for the same covariates as Model 1 (HR: 1.3, 95% CI: 1.1–1.5). Model 3 also considered acquired comorbidity but instead used multiply imputed Charlson index values and exercise status in place of missing longitudinal values (HR: 1.4, 95% CI: 1.2–1.6). We did not control for tumor and treatment characteristics because they are part of the causal pathway between comorbidity and mortality on our causal diagram (Fig. 1). Statistical adjustment for such variables would be expected to attenuate the observed hazard ratio by removing a portion of the total causal effect. We tested this expectation by additionally adjusting for stage, histologic grade, estrogen receptor status, surgery type, receipt of adjuvant tamoxifen, and receipt of adjuvant chemotherapy. Adjustment for these variables reduced the comorbidity hazard ratio in each of the 3 models by 5% or less.

DISCUSSION

We followed 689 breast cancer survivors for a median of 85 months; 33 months longer than a similar previous study.[8] We found that accounting only for baseline comorbidity gave approximately the same hazard ratio associating burden of comorbidity with all-cause mortality, compared with when comorbidity was regressed as a time-varying exposure (Hazard ratios: 1.4 and 1.3, respectively). Use of multiple imputation to populate missing values in longitudinal Charlson comorbidity data gave the same result (HR: 1.4). We consider our best estimate of the hazard ratio for a unit increase in Charlson index on the rate of all-cause mortality to be from Model 3, which used imputed values for all missing independent variables in the model. This estimate (HR: 1.4, 95% CI: 1.2–1.6) was consistent with the findings from an earlier study that examined the association between baseline Charlson index and all-cause mortality while controlling for age, primary treatment type, tumor stage, histologic grade, and hormone receptor status,[8] as well as a second study that examined only the impact of diabetes on all-cause mortality in breast cancer patients.[12]

Limitations

During our follow-up period, about 26% of subjects experienced an increase in comorbidity from baseline. Of those, approximately 80% had only a single-unit increase in Charlson index. These numbers indicate a relatively modest rate of comorbidity gain among cohort members. The present duration of follow-up—while the longest yet reported for a study of this association—may still be too short to capture an impact of longitudinal comorbidity on all-cause mortality rates. Our results indicate that a comorbidity assessment at the time of primary breast cancer treatment may provide sufficient short-term prognostic information (~7 years after surgery) for older breast cancer survivors.

Additional analyses with breast cancer mortality as the outcome would be of great interest. We could not conduct an appropriately powered analysis focused on breast cancer-specific mortality because the NDI registry does not yet contain cause of death data for all of the deceased subjects in our cohort.

The subjects in our cohort were predominately white (94%), so our results pertain mostly to women of that race. The poor representation of nonwhites in our cohort does not permit a rigorous evaluation of race/ethnicity as an effect modifier of the measured association between comorbidity and all-cause mortality.

Our results are susceptible to distortion by residual confounding, misclassification, and selection bias. Our exposure, outcome, and covariate data are subject to varying degrees of misclassification. Some subjects in our study were likely better historians of their medical history than others. There were 359 instances in which subjects failed to report one or more persisting medical condition in their 27, 51, and 87 month interviews, with respect to their baseline report. Of the 15 conditions that form the Charlson score, the most frequently under-reported at the 27-month interview—among those with a positive report for the condition at baseline—were heart failure (9%), diabetes (6%), stroke (5%), myocardial infarction (4%), connective tissue disease (4%), and pulmonary disease (3%). The remaining contributory conditions were under-reported with frequencies less than or equal to 2%. Under-reporting of medical history after the baseline interview was addressed by building monotonicity into the longitudinal Charlson index values. This method increased the sensitivity of comorbidity classification at the expense of specificity, which would cause overestimation of Charlson index if subjects falsely reported having certain conditions at any interview point. Although we cannot directly evaluate the extent of such overestimation in our own data, prior validation studies have shown that Charlson scores derived from interview data have test-retest reliability of approximately 0.9,[15] and are strongly correlated with scores derived from medical record review (correlation coefficient: 0.58, $P < 0.001$).[19] Our results did not differ substantially when we did not force monotonicity onto longitudinal Charlson index values, allowing them to decrease over time, indicating that our reported hazard ratios were not substantially affected by this potential source of misclassification of comorbidity.

Misclassification of confounders, if nondifferential with respect to outcome status, results in residual confounding.[20] Little, if any, misclassification is expected in the age and race/ethnicity variables, but exercise habits may be misreported by participants. Our measured exercise variable may also be an incomplete proxy for the conceptual entity for which we wished to control, which was routine physical activity that would affect the risk of both comorbid disease and all-cause mortality. The extent to which our measured exercise variable does not map to this concept informs the degree of residual confounding in our adjusted estimate of association. Adjustment for exercise decreased the crude hazard ratio associated with Charlson index by 2%, indicating a slight bias away from the null due to confounding by exercise habits. If exercise was nondifferentially misclassified in our data, 2% would be an underestimate of the true magnitude of the upward confounding bias and the true adjusted hazard ratio would be lower than what we observed. Validation studies of specific physical activity instruments have shown significant correlations between older subjects'

responses and objectively measured physiologic parameters, indicating regular physical activity[21,22] as well as with results from "gold standard" doubly-labeled water experiments.[23] Although our assessment of exercise habits relied on none of the particular instruments examined by the validation studies, our question to participants was detailed and specific in nature and should be of similar validity. Responses to this interview question are expected to conform reasonably well to the ideal concept for which we sought to adjust. We therefore do not expect residual confounding by exercise to be of a sufficient magnitude to explain our result completely.

Misclassification of vital status is unlikely, given the reliability of the National Death Index, Social Security Administration, and Centers for Medicare and Medicaid Services death indices. Approximately 94% of deaths in our cohort were ascertained from the NDI, which has consistently demonstrated high sensitivity and specificity (both nearly 100%) for vital status.[20,24] Approximately 5% of deaths were ascertained through the Social Security Administration database, which while inferior to the NDI, also exhibits favorable classification accuracy.[24] Proxy interviews and the CMS database contributed only 1 death each, and any flaws in these sources would not have substantially influenced our results.

We restricted our analytic sample to women in our cohort who had completed a baseline interview (3 months after primary breast cancer surgery). If completion of the baseline interview was an effect of both comorbidity (or its absence) and vital status, then selection bias could distort our observed association.[25] We believe the most likely scenario is that subjects with a greater comorbidity burden at enrollment were less likely to complete their 3-month interview, either because of their illness or because of death before the 3-month point. If this pattern is indeed the case, the selection bias would have the effect of lowering the observed association between comorbidity and all-cause mortality, and could not account for our result. In support of this pattern, we observed a 42% higher odds of dying among the cohort subjects who did not complete a baseline interview, compared with those who did (OR: 1.42, 95% CI: 1.00–2.00). Our cohort also experienced loss to follow-up, which resulted in missing data in the exposure (comorbidity) and confounder (exercise) data. The sensitivity of our observed results to these losses was tested by modeling with multiply imputed values replacing the missing fields. Multiple imputation is an attractive alternative to carrying forward prior observations in longitudinal analyses; it yields results that incorporate uncertainty about the imputed values into confidence intervals.[17] Examination of our survival analysis results (Table 3) shows that the confidence interval around the hazard ratio corresponding to the multiply imputed data is actually the narrowest, despite the additional uncertainty it contains. This counter-intuitive result is likely explained by the ability of this model to include 71 additional observations from subjects without baseline exercise data. Inclusion of these observations in the imputation model apparently increases precision more than the imputation decreases it.

In conclusion, we found that a unit increase in the Charlson index of comorbidity was associated with a 40% higher hazard of death from any cause among older survivors of early stage female breast cancer. The same general result was observed whether or not we accounted for acquired comorbidities and missing data. The modest rate of comorbidity gain in our cohort may be responsible for the equivalent results between longitudinal and baseline-only accounting of comorbidity. Additional prognostic value of longitudinal comorbidity may become evident upon longer follow-up.

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CANCER

Trends in breast-conserving surgery in Denmark, 1982–2002

Thomas P. Ahern · Heidi Larsson · Jens Peter Garne · Deirdre P. Cronin-Fenton · Henrik Toft Sørensen · Timothy L. Lash

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Abstract Using hospital discharge data from the counties in Northern Denmark and the Danish Cancer Registry, we examined the trend in the prevalence of breast-conserving surgery (BCS) to treat primary breast cancer from 1982 through 2002, with an emphasis on publications that may have influenced surgical practice in Denmark. Overall, the prevalence of BCS increased from less than 1% of breast cancer operations in 1982 to approximately 25% by 2002. The rise in prevalence was most pronounced for the treatment of young women and women with early-stage breast cancer. Of three pivotal clinical trials, the most significant trigger of the upward trend appeared to be a study conducted by the Danish Breast Cancer Cooperative Group, published in 1988. After 1988, there was a steep rise in the prevalence of BCS. By 2002, BCS prevalence appeared to reach a threshold at 25% of breast cancer operations, seemingly defined by the proportion of new breast cancer cases who are good candidates for BCS.

Keywords Epidemiology · Breast Neoplasms · Mastectomy · Segmental

T. P. Ahern (✉) · H. T. Sørensen · T. L. Lash
Department of Epidemiology, Boston University School of Public Health, 715 Albany Street, TE3, Boston, MA 02118, USA
e-mail: tpa@bu.edu

H. Larsson · D. P. Cronin-Fenton · H. T. Sørensen · T. L. Lash
Department of Clinical Epidemiology, Aarhus University Hospital, Ole Worms Allé 1150, 8000 Aarhus C, Denmark

J. P. Garne
Department of Surgical Oncology, Aalborg University Hospital, Hobrovej 18–22, PO Box 365, 9100 Aalborg, Denmark

Abbreviations

BCS	Breast-conserving surgery
BCS-RT	Breast-conserving surgery with radiotherapy
CPR	Civil personal registration number
DBCG	Danish Breast Cancer Cooperative Group
DCR	Danish cancer registry
HDR	Hospital discharge registry
ICD	International Classification of Diseases
NSABP	National Surgical Adjuvant Breast and Bowel Project
SEER	Surveillance, epidemiology and end results
UICC	International Union Against Cancer

Introduction

Before the 1980s, surgical treatment of breast cancer was almost entirely accomplished by Halsted's radical mastectomy procedure, first published in 1898, or modifications thereof [1]. Despite pervasive use of radical mastectomy to treat all types of breast cancer, sporadic case reports of breast-conserving surgery (BCS) appeared in the literature throughout the 1970s [1–3]. The motivation to develop this procedure apparently stemmed from increasing detection of breast cancer cases at early stages, for which the traditional radical mastectomy seemed an overtreatment [1]. The breast-conserving procedure was characterized by local excision of the tumour with follow-on radiotherapy to ablate occult tumour foci remaining in the breast. The first clinical trial comparing BCS and radiotherapy to mastectomy was published in 1972, and showed that BCS, compared to mastectomy, resulted in a higher incidence of local and distant recurrences as well as significantly reduced 10-year overall survival in

node-positive patients (Manchester stage 2) [4]. This result was later attributed to a naïveté regarding the importance of tumour-free tissue margins and an insufficient radiation dose (32 Gy) given in the BCS treatment regimen [1]. Preliminary results from subsequent trials comparing BCS coupled with radiotherapy (BCS-RT) to mastectomy were published in the 1980s and early 1990s, [5–8] with long-term updates appearing thereafter [9–13]. The combined evidence of these studies overwhelmingly supported equivalency of BCS-RT to mastectomy in patients with early stage breast cancer (UICC stage I or II) with respect to both disease-free and overall survival [5–13]. In addition, cosmetic and adverse effect outcomes were more favorable, on average, for patients undergoing BCS [5].

The aim of this descriptive study is to examine the trend in BCS prevalence among all primary treatment operations for patients with non-metastatic breast cancer in the northern part of Denmark from 1982 through 2002, with reference to important publication events that may have influenced the adoption of BCS over mastectomy during that time period.

Patients and methods

Landmark clinical trial ascertainment

A literature search was conducted to identify major clinical trial results comparing BCS to mastectomy, which were likely to influence surgical practice in Denmark. Three randomized clinical trials were deemed most influential to the Danish breast surgical community over the time period: a trial conducted at the Milan Cancer Institute and published in 1981 [7], a similar trial published in 1985 by the U.S. National Surgical Adjuvant Breast and Bowel Project (NSABP Protocol B-06) [6], and a trial conducted by the Danish Breast Cancer Cooperative Group (DBCG-82TM), published in 1988 [5]. Preliminary reports were issued from these trials in the 1980s, with periodic updates published throughout the remainder of the study period.

Breast cancer surgical data collection

We identified surgical procedures for women (either mastectomy or BCS) related to a diagnosis of breast cancer by linking the hospital discharge registries of three Danish counties (North Jutland, Viborg, and Aarhus; population 1.4 million) to the Danish cancer registry (DCR). A hospital discharge registry has been in operation in each Danish county since 1977 (in Viborg County since 1972) and records dates of admission and discharge, surgical procedure(s) performed, and up to 20 discharge diagnoses immediately after the discharge of the patient. Data from the hospital discharge registries from the three counties have been merged into a research database at Aarhus University, Denmark and linked to data from the DCR. Patients are identified in the databases by their civil personal registration (CPR) numbers, a unique number issued to all Danish residents at birth or emigration that encodes gender and birth date. The CPR number is used by all Danish registries and facilitated linkage of the hospital discharge registry data to the DCR for this study.

Using the HDR we identified the first operation sequence related to a breast cancer diagnosis for each woman in the register. An operation sequence was considered to be related to a breast cancer diagnosis if a diagnosis was given at the time of discharge, or if a diagnosis was registered in a separate admission record with a discharge date within 90 days of the surgical date. Breast cancer diagnoses and surgical procedures were classified in the HDR using ICD-8 (until 1993) and ICD-10 (from 1994 forward) codes. Complete data were available for the three counties from January 1, 1982 to December 31, 2002. No organized screening for breast cancer by mammography occurred in the study area during this period.

About 15,502 records meeting the inclusion criteria were identified, representing 14,487 women with a total of 15,605 operations. For women who received more than one breast cancer operation within a 60 day period (for instance, BCS followed by mastectomy), we defined the most recent procedure as the surgical treatment type. We excluded records for women with no registration in the DCR (N = 1,087), women whose treatment course did not fall entirely within the date ranges examined (N = 1,367), women whose first DCR registration was for bilateral cancer or if two unilateral cancers were recorded on the same date (N = 610), women with duplicate DCR records for the same breast (N = 14), women for whom more than 6 months had elapsed between the first operation and appearance of the DCR record (N = 236), and women whose surgical sequences were inconsistent (for instance, first operation being a mastectomy followed by a record of BCS), (N = 20). We also excluded records for women with metastatic disease, since the choice of operation type for these patients depends upon a different set of factors and clinical expectations than the choice for women with local or regional disease. After applying these exclusion criteria, 10,775 women remained in the analysis.

We obtained data on summary disease stage (classified as local, regional or metastatic) from the DCR. Summary staging is commonly employed by cancer registries and provides a broader categorization of disease characteristics than the clinical TNM staging systems. This feature of summary staging allowed us to evaluate trends within

levels of stage without using the more finely divided clinical staging categories. In certain cases, a summary stage cannot be reliably assigned either due to incomplete diagnostic data or contradictory reports. Out of the 10,775 women in the analysis, 480 (4.5%) were classified as unknown stage.

Data analysis

The prevalence of BCS among all breast cancer surgeries was computed for each year during the study period, based on the date of the definitive surgical procedure. Patient age at the time of surgery was categorized into approximate quartiles (20–49, 50–59, 60–69 and 70–100 years) based on the univariate distribution in the entire sample. One subject in the data set had a recorded age of 8 years and was excluded from the analysis.

Smoothed plots of BCS prevalance were generated by averaging the monthly proportions across a 5-month window, and advancing this window 1 month at a time. Proportions at the center of the window were weighted more heavily than proportions at the window edges. These smoothed proportions were divided into four time periods defined by the intervals between (A) publication of the Milan and NSABP studies, (B) publication of the NSABP and DBCG studies, (C) publication of the DBCG study and the first report of fraud within the NSABP trial [10], and (D) the report of fraud and the end of the study period. The trend within each time interval was fit with a cubic spline function generated by maximizing the binomial likelihood of the observations within the interval [14]. These plots were generated for the crude trend as well as within strata of stage (local and regional) and strata of age (20–49, 50–69 and 70–100 years). Markers indicating the publication year of major clinical trial results comparing BCS-RT to mastectomy were included on all of the plots. Differences in the distribution of age and stage categories between surgical groups were assessed by Pearson's chi-squared tests. Two-sided *P*-values testing the null hypothesis are reported. All analyses were performed with the SAS version 9.1 (SAS Institute, Cary, North Carolina, USA).

Results

Landmark clinical trials

The first of three definitive clinical trials comparing BCS to mastectomy was conducted at the National Cancer Institute in Milan, Italy. It randomized women with stage I breast cancer (tumour diameter $\leq$ 2 cm) either to Halsted radical mastectomy or to quadrantectomy with combination radiotherapy [7]. After 7 years of follow-up, the trial data showed similar disease-free and overall survival rates for the two treatment groups, with fewer post-operative complications reported in the BCS group [7].

The second trial, performed by the U.S. National Surgical Adjuvant Breast Project, compared total mastectomy to segmental mastectomy, with our without radiation therapy. The segmental mastectomy procedure was considerably more conservative than the quadrantectomy used in the Milan study; it stipulated tissue resection only to the extent that excised specimen margins were free of tumour. The NSABP trial reached the same main conclusion as the Milan trial; mastectomy and BCS-RT were equivalent with respect to disease-free and overall survival. More importantly, the trial results provided strong evidence that radiotherapy is of great additional benefit to reduce recurrence risk among those subjects undergoing BCS, regardless of their age, nodal status and tumour size.

The third trial was conducted by the Danish Breast Cancer Cooperative Group (DBCG-82TM) [5], when the surgical standard for breast cancer treatment in Denmark solely consisted of total mastectomy with dissection of lower axillary lymph nodes. Patients either received standard mastectomy or a BCS procedure similar to that employed in the NSABP trial. All subjects who underwent BCS received postoperative radiotherapy. The trial's preliminary results, published in 1988 after 6 years of follow-up, indicated equivalence of BCS-RT to mastectomy with respect to recurrence-free survival. The results also indicated that approximately 25% of newly diagnosed breast cancer cases at the participating clinics were candidates for BCS.

Trend in breast-conserving surgery

Of the 10,775 women in the data set, 1,461 (13.6%) underwent BCS. Distributions of age and disease stage between the mastectomy and BCS groups are shown in Table 1. The median age of the women who received BCS was 8 years younger than that of the women who received mastectomy (53.8 and 61.9 years, respectively). As would be expected, women who received BCS were more likely to have less-advanced disease than women who received mastectomy.

Figure 1 shows the overall upward trend in the proportion of breast-conserving procedures in the three Danish counties between 1982 and 2002. These crude data are derived from 10,772 records, and the total number of breast cancer operations performed each year ranged from 334 to 759. There are two small increases in the prevalence of BCS, which are seen in the data points but are not reflected in the smoothed curve, following publication of both the

Table 1 Characteristics of surgical treatment groups, *N* (row %)

	Mastectomy *N* = 9,313	Breast-conserving *N* = 1,462	*P*-value[a]
Age (years)			
20–49	2,174 (80.0)	544 (20.0)	<0.0001
50–59	2,086 (82.7)	438 (17.4)	
60–69	2,197 (88.8)	276 (11.2)	
70–100	2,856 (93.3)	204 (6.7)	
Summary disease stage			
Local	4,738 (83.4)	945 (16.6)	<0.0001
Regional	4,148 (89.9)	465 (10.1)	
Unknown	428 (89.2)	52 (10.8)	

[a] Two-sided *P*-values from Pearson's chi-square test, $\alpha = 0.05$

Milan and NSABP trials, with a more considerable upward jump following publication of the DBCG trial's preliminary results. Over the study period, overall BCS prevalence rose from 0.9% in 1982 to 25.2% in 2002.

The stage-stratified trend plot shown in Fig. 2 shows a steeper rise in prevalence of BCS for the treatment of local stage disease compared to the trend for regional disease. Both curves start at 1982 with approximately 1% prevalence; they begin to diverge following publication of the preliminary NSABP results in 1985. In 2002, BCS prevalence was 31.7% and 18.7% in the local and regional strata, respectively.

The most striking difference in BCS trend was seen across age categories, as shown in Fig. 3. A pronounced jump in the use of breast-conserving procedures for subjects aged 20–49 was seen immediately following publication of preliminary results from DBCG-82TM. A similar though somewhat attenuated trend was seen for women aged 50–69, whereas the trend for women aged 70–100 remained relatively flat. In 2002, BCS prevalence was 27.9% among women aged 20–49, 28.4% among women aged 50–69, and 16.7% among women aged 70–100. When the youngest age stratum was restricted to women with local disease, the proportion receiving BCS in 2002 increased to 32.5% (data not shown). Since the presence of axillary metastases is strongly correlated with tumour size, and because tumour size is a stronger determinant than axillary involvement in the choice of BCS, this finding likely reflects an effect of smaller tumour size (not an effect of axillary involvement) on the use of BCS.

Discussion

To achieve adequate margins and an acceptable cosmetic result in BCS, the relative size of the tumour to the breast and the location of the tumour in the breast are important factors. The smaller the tumour, the greater the possibility for BCS. In the counties included in the study, no organized screening by mammography occurred during the study period and it is unlikely that the observed increase in BCS prevalence was caused by an increase in patient-requested mammography. Rather, it is likely the result of an increasing acceptance of the procedure among surgeons and patients. It is also possible that BCS may have been widely introduced in Denmark earlier, had it not been that the majority of Danish surgeons were awaiting the results of the DBCG-82TM trial, in which many of them participated.

In 1991, 2 years after publication of 8-year follow up results of the NSABP trial, the NSABP verified that falsified data had been reported by St. Luc Hospital in

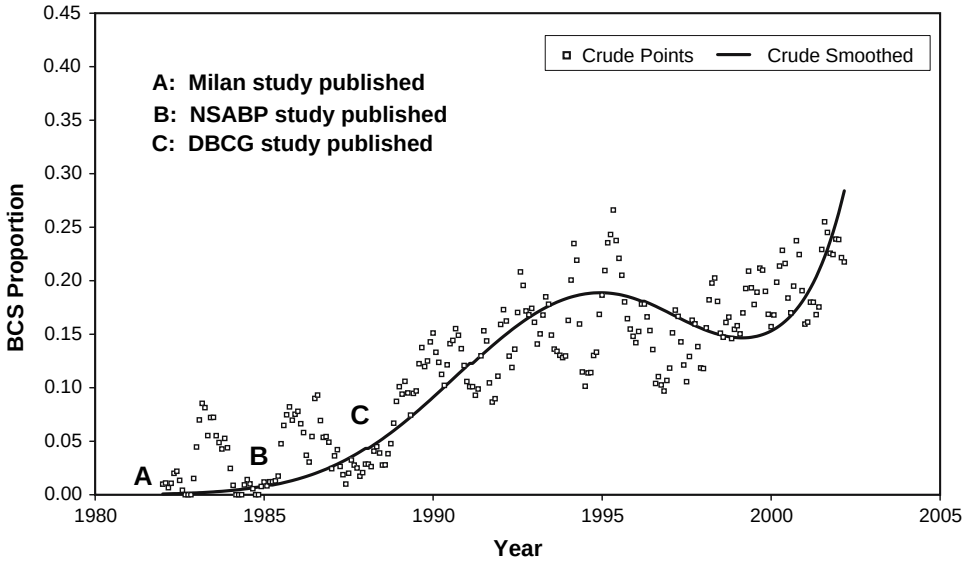

Fig. 1 Overall trend in breast-conserving surgery prevalence in North Jutland, Viborg and Aarhus counties, Denmark, 1982–2002

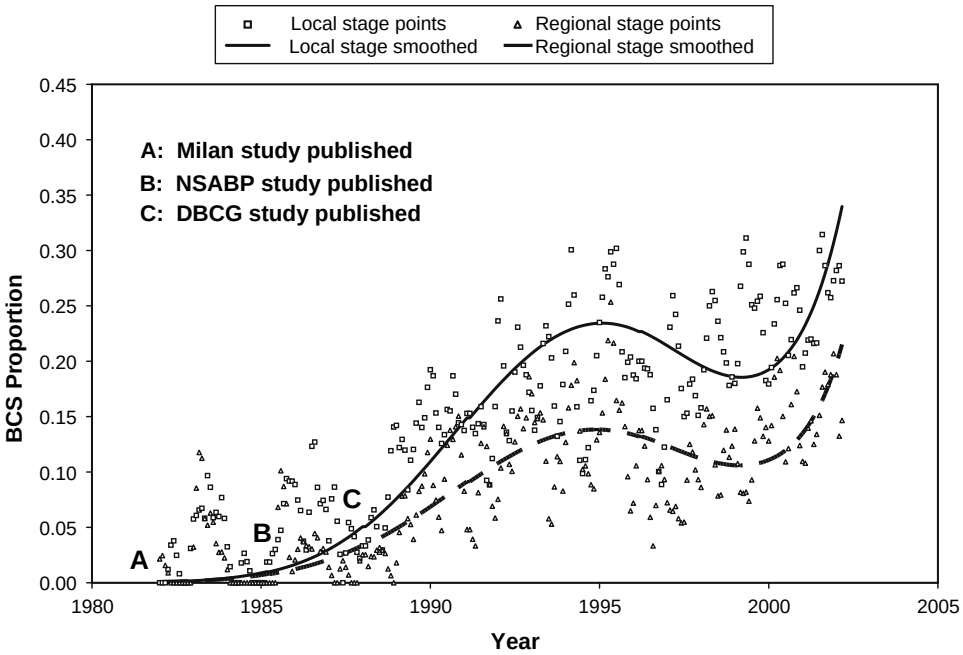

Fig. 2 Trend in breast-conserving surgery prevalence for local and regional disease stages, 1982–2002

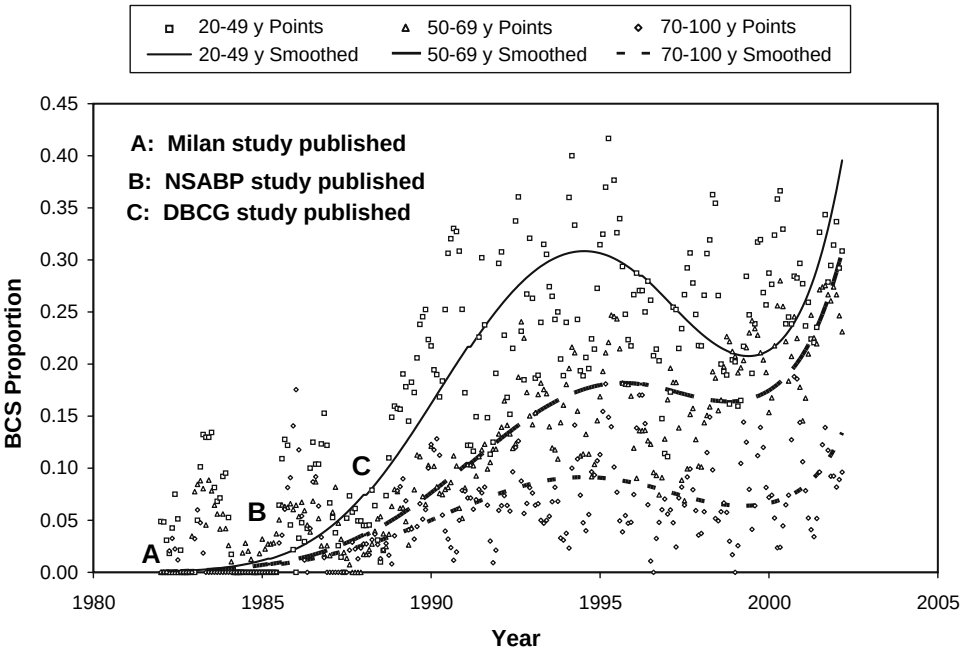

Fig. 3 Trend in breast-conserving surgery in women aged 20–49, 50–69 and 70–100 years

Montreal, a participating site in the B-06 study. Saint Luc Hospital had enrolled a total of 354 research subjects, six of whom had false biopsy dates reported to the NSABP headquarters. The report of fraud received considerable media attention and was followed by an extensive audit of the NSABP Protocol B-06 data in 1994, which uncovered no further corruption of data. The exonerated investigators re-analyzed the trial results with and without the fraudulent subjects included and published their results along with 12-year follow up data in 1995; conclusions from both analyses did not differ, again confirming the equivalence of BCS-RT to mastectomy [10]. Whether these events impacted on the dip in BCS prevalence around 1995 is not testable but is worth noting, especially since the trend resumed its climb following publication of the confirmed results.

The authors of the first report from DBCG-82TM noted that approximately 25% of the incident breast cancer cases presenting to the participating clinics were eligible for BCS [5]. This figure matches the overall prevalence of BCS in the three Danish counties in 2002. Twenty-five percent may be a "prevalence ceiling" for BCS, restricted by the

proportion of eligible cases in the population, and may have been reached by that year. If this conclusion is correct, then no further rise in the prevalence of BCS can be expected unless more pervasive mammographic screening programs for asymptomatic women are successfully initiated in Denmark, whereby cases will be detected in younger women and at earlier disease stages. Recent legislation to promote such screening practices by 2008 may lead to a future increase in the prevalence of BCS. In Sweden, where nationwide coverage for screening mammography began in 1991 [15], the overall prevalence of BCS increased from 7% in 1980 to 18% in 1985, and was 51% in 1995, 4 years after the initiation of screening mammography [16]. In the United States, where surveillance mammography became widespread in the 1980s, the prevalence of BCS rose from approximately 30% in 1988 to approximately 60% by 1998 [17].

Our study sample was the combined populations of North Jutland, Viborg and Aarhus counties (1.4 million people; approximately 26% of the total population of Denmark). While a larger sample would have yielded more precise prevalence estimates, the actual estimates and their trend over time in the whole of Denmark should be accurately depicted by the three chosen counties. The Danish Breast Cancer Cooperative Group was established in 1976 to ensure optimal diagnosis and treatment of operable primary breast cancer on a nationwide basis [18]. Because the DBCG directs breast cancer treatment protocols on a national level, there is no reason to suspect substantially different treatment patterns by geographic region.

In conclusion, the prevalence of BCS increased from approximately 1 to 25% of all breast cancer operations in Viborg, North Jutland and Aarhus counties in Denmark during the period 1982–2002. The prevalence rose most considerably following publication of initial results from a Danish clinical trial comparing BCS to mastectomy, which showed equivalence of the two procedures with respect to recurrence-free survival among women with invasive breast carcinoma. By 2002, the prevalence appeared to reach a plateau, perhaps defined by the proportion of breast cancer cases in the Danish population who are good candidates for BCS.

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Full Paper

Tamoxifen's protection against breast cancer recurrence is not reduced by concurrent use of the SSRI citalopram

TL Lash*[1,2,3], L Pedersen[2], D Cronin-Fenton[2], TP Ahern[1], CL Rosenberg[3], KL Lunetta[4], RA Silliman[3], S Hamilton-Dutoit[5], JP Garne[6,7], M Ewertz[7,8] and HT Sørensen[1,2,7]

[1]Department of Epidemiology, Boston University School of Public Health, 715 Albany Street, Boston, MA 02118, USA; [2]Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43, Aarhus N 8200, Denmark; [3]Department of Medicine, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA; [4]Department of Biostatistics, Boston University School of Public Health, 715 Albany Street, Boston, MA 02118, USA; [5]Institute of Pathology, Aarhus University, Noerrebrogade 44, Aarhus C 8000, Denmark; [6]Department of Surgery, Aalborg Hospital, Aarhus University Hospital, Hobrovej 18-22, Aalborg 9100, Denmark; [7]On behalf of the Danish Breast Cancer Co-operative Group (DBCG); [8]Department of Oncology, Odense University Hospital, Sdr. Boulevard 29, Odense C 5000, Denmark

Tamoxifen remains an important adjuvant therapy to reduce the rate of breast cancer recurrence among patients with oestrogen-receptor-positive tumours. Cytochrome P-450 2D6 metabolises tamoxifen to metabolites that more readily bind the oestrogen receptor. This enzyme also metabolises selective serotonin reuptake inhibitors (SSRI), so these widely used drugs – when taken concurrently – may reduce tamoxifen's prevention of breast cancer recurrence. We studied citalopram use in 184 cases of breast cancer recurrence and 184 matched controls without recurrence after equivalent follow-up. Cases and controls were nested in a population of female residents of Northern Denmark with stages I–III oestrogen-receptor-positive breast cancer 1985–2001 and who took tamoxifen for 1, 2, or most often for 5 years. We ascertained prescription histories by linking participants' central personal registry numbers to prescription databases from the National Health Service. Seventeen cases (9%) and 21 controls (11%) received at least one prescription for the SSRI citalopram while taking tamoxifen (adjusted conditional odds ratio = 0.85, 95% confidence interval = 0.42, 1.7). We also observed no reduction of tamoxifen effectiveness among regular citalopram users (⩾30% overlap with tamoxifen use). These results suggest that concurrent use of citalopram does not reduce tamoxifen's prevention of breast cancer recurrence.

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Tamoxifen is a selective oestrogen receptor modulator (Jordan and Dowse, 1976) that reduces by half the risk of breast cancer recurrence in early-stage patients whose tumour cells express the oestrogen receptor (Early Breast Cancer Trialists' Collaborative Group, 2005). To be pharmacologically active, tamoxifen must be metabolised to secondary metabolites that bind the oestrogen receptor 100-fold more readily than tamoxifen itself (Malet *et al*, 1988). Four cytochrome P-450 enzymes (CYPs) catalyse this activation (CYP2D6, CYP3A4, CYP3A5, and CYP2C9) (Malet *et al*, 1988). CYP2D6 catalyses formation of 4-hydroxytamoxifen from tamoxifen (Coller *et al*, 2002) and formation of 4-hydroxy-*N*-desmethyltamoxifen from *N*-desmethyltamoxifen (Stearns *et al*, 2003). These two secondary metabolites have the highest binding affinity for the oestrogen receptor, and binding affinity correlates with inhibition of cell growth (Coezy *et al*, 1982). The secondary metabolites are, therefore, the most important modulators of the oestrogen receptor in the tamoxifen pathway (Lim *et al*, 2005).

*Correspondence: Dr TL Lash, Department of Epidemiology, Boston University School of Public Health, 715 Albany St., TE3, Boston, MA 02118, USA; E-mail: tlash@bu.edu
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Breast cancer patients treated with tamoxifen may also take other prescription medications that are metabolised by some of the same enzymes that activate tamoxifen. For example, depression is a common comorbidity in breast cancer patients (Massie, 2004), and many selective serotonin reuptake inhibitors (SSRI), which are widely used medications indicated primarily to treat depression (Hansen *et al*, 2003), are metabolised by CYP2D6 (Zanger *et al*, 2004). SSRI competition with tamoxifen and *N*-desmethyltamoxifen for CYP2D6, or direct inhibition of CYP2D6 by SSRI, could reduce the production of the tamoxifen metabolites with high receptor-binding affinity, and thereby reduce tamoxifen's prevention of breast cancer recurrence. Competition between tamoxifen and the SSRI paroxetine reduced the plasma concentration of endoxifen in a cross-over clinical trial (Stearns *et al*, 2003). Furthermore, the mean plasma concentration of 4-hydroxy-*N*-desmethyltamoxifen was more than two-fold greater among women who were taking no CYP2D6 competitor drug than among women who were taking such a drug (Jin *et al*, 2005). *In vivo* studies thus demonstrate a compelling biological basis for the hypothesis that concomitant use of SSRI would reduce tamoxifen's prevention of breast cancer recurrence.

In the largest study to date of the potential for drug–drug interaction to reduce tamoxifen's protection against breast cancer

recurrence, we examined whether Danish breast cancer patients with oestrogen-receptor-positive tumours who were treated with tamoxifen for 1, 2, or most often for 5 years had a higher rate of recurrence if they were concomitantly taking the SSRI citalopram or its S-stereoisomer ('citalopram' from here onwards) than if they were not. As described in more detail below, citalopram was the most frequently prescribed SSRI in the study population.

MATERIALS AND METHODS

The study was approved by the Boston University Medical Campus Institutional Review Board and The Regional Committee on Biomedical Research Ethics of Aarhus County.

Study population

The source population included female residents of four Northern Danish counties (Aarhus, North Jutland, Viborg, and Ringkøbing) aged 35–69 at diagnosis of primary International Union Against Cancer stage I, II, or III breast cancer (UICC, 1997) between 1985 and 2001 and who were reported to the Danish Breast Cancer Cooperative Group (DBCG). The DBCG has enrolled nearly all Danish breast cancer patients younger than age 70 at diagnosis into its clinical database since 1977 (Andersen and Mouridsen, 1988; Jensen *et al*, 2003). More than 90% of Danish breast cancer cases are reported to the DBCG and more than half of the DBCG patients are enrolled in clinical trials (Andersen and Mouridsen, 1988). The same standardized forms are used to follow all patients reported to the DBCG, regardless of whether they enrol in a trial, so the registry provides the data quality advantage of a clinical trial setting with the generalisability advantage of a population-based setting.

We divided the source population into three groups: (a) group I women whose tumour expressed the oestrogen receptor protein and who were treated with tamoxifen for at least 1 year; (b) group II women whose tumour did not express the oestrogen receptor protein, were not treated with tamoxifen, and who survived for at least one year; and (c) group III women, comprising all others, who were excluded from this analysis. Group I women were assigned to tamoxifen therapy protocols of 1, 2, or 5 years, depending on the guideline extant in Denmark at the time of their diagnoses. We included group II women to estimate the direct association of citalopram prescription with recurrence rate, if any. We further restricted the source population to women diagnosed with breast cancer after the date that their county of residence began to maintain an electronic prescription database (Aarhus = 1996, North Jutland = 1989, Ringkøbing = 1998, Viborg = 1998), which were used to ascertain use of prescription medications, including citalopram. Follow-up time began 1 year after the date of breast cancer diagnosis and continued until the date of the first of breast cancer recurrence, death from any cause, loss to follow-up (e.g., emigration), 10 years of follow-up, or 1 September 2006.

Cases were women with local or distant breast cancer recurrence occurring during their follow-up time among the members of groups I and II. We selected one control for each case without replacement from members of the source population who had not had a breast cancer recurrence after the same amount of follow-up time. We matched controls to cases on (a) group membership (group I or II), (b) menopausal status at diagnosis (premenopausal or postmenopausal), (c) date of breast cancer surgery (caliper matched ± 12 months), (d) county of residence at the time of diagnosis, and (e) UICC stage at diagnosis (stage I, II, or III).

Data collection

We used the Danish Civil Personal Registration (CPR) number assigned to each case and control to link data sets. The CPR is a unique identification number assigned to all Danish residents alive on 1 April 1968, born thereafter, or upon immigration.

We collected demographic information (age, menopausal status, and hospital of diagnosis), tumour characteristics (UICC stage, histological grade, and oestrogen-receptor expression), and therapy characteristics (primary surgical tumour management, receipt of radiation therapy, receipt of chemotherapy, and receipt of tamoxifen therapy) from the DBCG database.

We collected data on receipt of citalopram prescription and other potential CYP2D6 inhibitors (including other SSRI) by linking the CPR number of cases and controls to the prescription databases maintained by each county (see, for example, the description of North Jutland's database (Gaist *et al*, 1997)).

Analytic variables

Recurrence We used the DBCG definition of breast cancer recurrence as any type of breast cancer subsequent to the initial course of therapy (Andersen and Mouridsen, 1988). Given the definition of the source population and follow-up time, all cases of recurrence occurred between 1 and 10 years after the primary breast cancer diagnosis.

Prescription status Prescription medications are coded by the Anatomical Therapeutic Chemical (ATC) classification system (WHO Collaborating Centre for Drug Statistics Methodology, 2007). We defined SSRI antidepressants as all those classified in group N06AB by the ATC. These are the SSRI drugs: zimeldine (N06AB02), fluoxetine (N06AB03), citalopram (N06AB04), paroxetine (N06AB05), sertraline (N06AB06), alaproclate (N06AB07), fluvoxamine (N06AB08), etoperidone (N06AB09), and escitalopram (N06AB10). We defined citalopram exposure as any prescription for citalopram (N06AB04) or its S-stereoisomer escitalopram (N06AB10).

We classified cases and controls as those with no record of a citalopram prescription during their follow-up time (never citalopram) and those with any record of prescription for citalopram during their follow-up time (ever citalopram). We used a similar procedure to classify cases and controls as ever or never users of another SSRI or of another prescription medication that is a CYP2D6 inhibitor or substrate, aside from those indicated to treat breast cancer recurrence or its effects. See the Supplementary online material for a complete list of these medications and the frequency of their use in the study population.

For group I women who ever had a citalopram prescription, we calculated the percentage of time on tamoxifen when they were simultaneously taking citalopram. We created categories of (a) intermittent citalopram use, defined as citalopram use overlapping tamoxifen use for more than 0% but less than 30% of the time on tamoxifen and (b) regular citalopram use, defined as citalopram use overlapping tamoxifen use for 30% or more of the time on tamoxifen. We chose 30% as the overlap boundary to allow sufficient sample size in the regular citalopram subgroup, while also investigating a substantial period of SSRI and tamoxifen comedication.

Covariates We defined the following set of covariates: (a) time period of breast cancer diagnosis (1985–1993, 1994–1996, and 1997–2001), (b) age at diagnosis (35–44 years, 45–54 years, 55–64 years, and 65–70 years), (c) menopausal status at diagnosis (premenopausal and postmenopausal), (d) county of residence at diagnosis (Aarhus, North Jutland, Viborg, and Ringkøbing), (e) UICC stage at diagnosis (stages I, II, and III), histological grade (grade I, II, III, and missing), surgery type (breast conserving surgery and mastectomy), and receipt of systemic adjuvant chemotherapy (yes and no), and (f) receipt of a prescription for another medication that is a CYP2D6 inhibitor or substrate, including other SSRI, while taking tamoxifen.

Analytic strategy

All analyses were conducted within strata of the two groups (oestrogen-receptor positive and treated with tamoxifen or oestrogen-receptor negative and not treated with tamoxifen). We computed the frequency and proportion of cases and controls within categories of assigned protocol of tamoxifen duration, of citalopram use, of use of other CYP2D6 inhibitors or substrates, and of the covariates. We calculated the number of cases and controls ever receiving citalopram, the number of total prescriptions for citalopram summed over all cases or controls, and the range of the number of prescriptions for citalopram received by each individual case or control.

We estimated the rate ratio associating citalopram prescription with breast cancer recurrence as the odds ratio (OR) in a conditional logistic regression including only citalopram use as the exposure variable and conditioned on the matched factors. By design, this ratio adjusts for confounding by the matched factors (Greenland, 2008). We examined whether the effect of citalopram use was modified by duration of tamoxifen therapy in a stratified analysis. Finally, we adjusted for residual confounding by the covariates that were not included in the matching by including them as independent variables in the conditional logistic regression. We retained in the final model any covariate that affected the log OR from the conditional logistic regression model associating citalopram use with breast cancer recurrence rate by more than 10% (Greenland, 1989). All estimates are accompanied by a 95% confidence interval (CI) calculated by the profile likelihood method. All analyses were performed using SAS version 9.

RESULTS

Table 1 shows the frequency and proportion of cases and controls, within strata of group, in the categories of the covariates. About two-thirds of cases and controls in both groups were diagnosed with primary breast cancer during the period 1997–2001, and the majority was resident in Aarhus or North Jutland counties, because the prescription registries began first in these two counties. A large majority had mastectomy as their primary surgical intervention, which is consistent with the clinical practice pattern previously reported in this region during this time period (Ahern *et al*, 2008). Group I women (positive oestrogen-receptor expression and treated with tamoxifen) were more likely to be post-menopausal (87%) than were group II women (66%; negative oestrogen-receptor expression and not treated with tamoxifen). Group I women were also less likely to receive systemic adjuvant chemotherapy (11 and 13% of cases and controls, respectively) than were group II women (80 and 70% of cases and controls, respectively); reflecting the preference for hormonal therapy over systemic adjuvant chemotherapy in women whose tumours expressed the oestrogen receptor. Between 3 and 11% of cases and controls ever used citalopram while taking tamoxifen (group I) or during their follow-up period (group II).

Table 2 depicts the pattern of SSRI prescriptions received by cases and controls. In both groups, SSRI prescriptions were primarily written for citalopram or its S-stereoisomer, escitalopram. For example, 17 of 23 group I cases (74%) ever prescribed an SSRI had at least one prescription for citalopram, accounting for 86% of the total number of prescriptions. Similarly, 22 of 30 group I controls (73%) ever prescribed an SSRI had at least one prescription for citalopram, accounting for 64% of their prescriptions. Sertraline accounted for the majority of the remaining prescriptions (11% of the total for cases and 23% for controls).

Group I women who ever used citalopram while taking tamoxifen did not have a higher rate of breast cancer recurrence than women who never used citalopram while taking tamoxifen (Table 3; OR = 0.79, 95% CI = 0.40, 1.6). This OR was not substantially modified by duration of tamoxifen therapy

Table 1 Frequency and proportion of cases of breast cancer recurrence and matched controls within group strata (I) expressing the oestrogen receptor and receiving at least 1 year of tamoxifen therapy (ERP+/TAM+), or (II) not expressing the oestrogen receptor, never receiving tamoxifen therapy, and surviving at least 1 year after diagnosis (ERP−/TAM−)

	Group I: ERP+/ TAM+ (*n* (%))		**Group II: ERP−/ TAM− (*n* (%))**	
	Cases	**Controls**	**Cases**	**Controls**
Citalopram prescription				
Ever	17 (9)	21 (11)	3 (3)	5 (6)
Never	167 (91)	163 (89)	84 (97)	82 (94)
Other CYP2D6 inhibitors, including other SSRI				
Ever	48 (26)	51 (28)	25 (29)	17 (20)
Never	136 (74)	133 (72)	62 (71)	70 (80)
Diagnosis year[a]				
1985–1993	33 (18)	34 (18)	13 (15)	11 (13)
1994–1996	32 (17)	29 (16)	17 (20)	18 (21)
1997–2001	119 (65)	121 (66)	57 (66)	58 (67)
Age at diagnosis				
35–44	13 (7)	11 (6)	15 (17)	12 (14)
45–55	38 (21)	34 (18)	37 (43)	29 (33)
55–65	91 (49)	93 (51)	26 (30)	29 (33)
65–70	42 (23)	46 (25)	9 (10)	17 (20)
Menopausal status at diagnosis[a]				
Premenopausal	24 (13)	24 (13)	30 (34)	30 (34)
Postmenopausal	160 (87)	160 (87)	57 (66)	57 (66)
County of residence at diagnosis[a]				
Aarhus	70 (38)	70 (38)	37 (43)	37 (43)
North Jutland	88 (48)	88 (48)	37 (43)	37 (43)
Viborg	15 (8)	15 (8)	9 (10)	9 (10)
Ringkøbing	11 (6)	11 (6)	4 (5)	4 (5)
UICC tumour stage at diagnosis[a]				
Stage I	7 (4)	7 (4)	4 (5)	4 (5)
Stage II	79 (43)	79 (43)	41 (47)	41 (47)
Stage III	98 (53)	98 (53)	42 (48)	42 (48)
Histological grade				
Grade I	31 (17)	33 (18)	4 (5)	17 (20)
Grade II	73 (40)	87 (47)	29 (33)	2 (2)
Grade III	44 (24)	24 (13)	38 (44)	22 (25)
Missing	36 (20)	40 (22)	16 (18)	46 (53)
Surgery type				
Breast conserving surgery	22 (12)	22 (12)	9 (10)	4 (5)
Mastectomy	162 (88)	162 (88)	78 (90)	83 (95)
Radiation therapy				
Yes	86 (47)	79 (43)	43 (49)	36 (41)
No	98 (53)	105 (57)	44 (51)	51 (59)
Tamoxifen protocol				
1 year	57 (31)	57 (31)	Not applicable	Not applicable
2 years	10 (5.4)	10 (5.4)		
5 years	117 (64)	117 (64)		
Systemic adjuvant chemotherapy				
Yes	21 (11)	24 (13)	70 (80)	61 (70)
No	163 (89)	160 (87)	17 (20)	26 (30)

[a]Variable included in risk set sampling to match controls to cases.

Table 2 Number of cases and controls receiving any prescription for each SSRI, and total number of prescriptions for each SSRI within group strata (I) expressing the oestrogen receptor and receiving at least 1 year of tamoxifen therapy (ERP+/TAM+), or (II) not expressing the oestrogen receptor, never receiving tamoxifen therapy, and surviving at least 1 year after diagnosis (ERP−/TAM−)

	Group I: ERP+/TAM+ *n* (no. of prescriptions) [range of no. per person]		**Group II: ERP−/TAM− *n* (no. of prescriptions) [range of no. per person]**	
SSRI name (ATC code)	**Cases**	**Controls**	**Cases**	**Control**
Zimeldine (N06AB02)	0	0	0	0
Fluoxetine (N06AB03)	1 (4) [4–4]	4 (24) [1–13]	2 (12) [1–11]	0
Citalopram (N06AB04)[a]	16 (251) [1–53]	21 (123) [1–24]	3 (4) [1–2]	5 (64) [1–43]
Paroxetine (N06AB05)	2 (6) [1–5]	1 (1) [1–1]	1 (2) [2–2]	3 (20) [5–9]
Sertraline (N06AB06)	3 (32) [3–24]	7 (45) [1–15]	1 (2) [2–2]	1 (12) [12–12]
Alaproclate (N06AB07)	0	0	0	0
Fluvoxamine (N06AB08)	0	0	0	0
Etoperidone (N06AB09)	0	0	0	0
Escitalopram (N06AB10)[a]	1 (2) [2–2]	1 (3) [3–3]	0	0

[a]In the analysis, we defined citalopram exposure as any prescription for citalopram (N06AB04) or its S-stereoisomer escitalopram (N06AB10).

Table 3 Association between SSRI prescription and breast cancer recurrence within strata of (a) Group I, women with tumours that expressed the oestrogen receptor and who received at least 1 year of tamoxifen therapy (ERP+/TAM+) or (b) Group II, women with tumours that did not express the oestrogen receptor, who never received tamoxifen therapy, and who survived at least 1 year after diagnosis (ERP−/TAM−)

Citalopram prescription	**Cases/controls**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)[a]**
(a) Group I: ERP+/TAM+			
Never user	167/163	1.0 (reference)	1.0 (reference)
Ever user	17/21	0.79 (0.40, 1.6)	0.85 (0.42, 1.7)
Intermittent use	10/14	0.69 (0.30,1.6)	0.72 (0.30, 1.7)
Regular use	7/7	0.97 (0.34, 2.8)	1.1 (0.37, 3.3)
(b) Group II: ERP−/TAM−			
Never user	84/82	1.0 (reference)	1.0 (reference)
Ever user	3/5	0.60 (0.14, 2.5)	0.78 (0.17, 3.6)

[a]Adjusted for age category and other CYP2D6-inhibiting medications (see the Supplementary online material for a complete list of these medications and the frequency of their use in the study population).

($P = 0.23$ for test of homogeneity; data not shown). The approximately null effect persisted with adjustment for age category and ever/never use of another CYP2D6 inhibitor or SSRI (OR = 0.85, 95% CI 0.42, 1.7). The effects were likewise approximately null within cumulative citalopram prescription categories (intermittent use OR = 0.72, 95% CI 0.30, 1.7; regular use OR = 1.1, 95% CI 0.37, 3.3). Citalopram use also had no substantial effect on recurrence in group II women (adjusted OR = 0.78, 95% CI 0.17, 3.6), suggesting that citalopram does not directly affect the risk of breast cancer recurrence.

DISCUSSION

The results of this study do not support the hypothesis that citalopram, taken concurrently with tamoxifen, reduces tamoxifen's protective effect against breast cancer recurrence in early-stage patients whose tumour cells express the oestrogen receptor.

Our results extend the findings from an earlier study of 28 stage II and III breast cancer patients with recurrence and their matched controls at a single United States oncology centre, which also reported no substantial modification of tamoxifen effectiveness by concomitant use of SSRI inhibitors of CYP2D6 (Lehmann *et al*, 2004). These results may seem at odds with the strong biological rationale and *in vivo* evidence that support the hypothesis that CYP2D6 inhibition would reduce tamoxifen's prevention of breast cancer recurrence. It is possible, however, that SSRI medications could reduce the plasma concentration of tamoxifen's secondary metabolites without reducing its anti-tumorigenicity (Ponzone *et al*, 2004; Ratliff *et al*, 2004; Stearns *et al*, 2004). Tamoxifen doses as much as 20-fold lower than the typical US dose of 20 mg day^{-1} affect biomarkers of cardiovascular, bone, and tumour end points (Decensi *et al*, 1998, 2003), so the approximately three-fold reduction in the plasma concentration of tamoxifen's secondary metabolites associated with concomitant receipt of the SSRI paroxetine (Jin *et al*, 2005) may have little consequence.

The key mechanistic question may be whether reduced concentrations of active tamoxifen metabolites result in substantially reduced occupancy of the oestrogen receptor. Dowsett and Haynes (2003) estimated that, in postmenopausal women on a daily dose of 20 mg tamoxifen, tamoxifen and its metabolites occupy 9994 of 10 000 oestrogen receptors. Replicating their calculation using the plasma concentrations of tamoxifen and its metabolites in women with no *CYP2D6* variant allele (Jin *et al*, 2005), tamoxifen and its metabolites would occupy 9999 of 10 000 receptors in women not taking any SSRI and 9997 of 10 000 receptors in women taking the strong *CYP2D6*-inhibiting SSRI paroxetine. Steady-state concentrations of tamoxifen and its metabolites may be sufficient to manifest fully tamoxifen's antitumorigenic effect in postmenopausal women regardless of whether *CYP2D6* inhibition reduces the concentration of some tamoxifen metabolites.

Nonetheless, our results should be considered with the following limitations in mind. First, the majority of SSRI prescriptions in our study were for citalopram or its S-stereoisomer, both originally manufactured by Lundbeck, a company headquartered in Denmark. Citalopram is a modest inhibitor of *CYP2D6* compared with some other SSRI medications (Jeppesen *et al*, 1996). These more potent inhibitors may reduce tamoxifen's protection against breast cancer recurrence, but their interaction with tamoxifen would not have been well measured by this study.

Second, we have not collected genotype data to characterize functional *CYP2D6* variants (Hayhurst *et al*, 2001) that affect the metabolism of tamoxifen (Jin *et al*, 2005). The combination of genotype and receipt of *CYP2D6*-inhibiting medications has been related to tamoxifen effectiveness in a previous study (Goetz *et al*, 2007). We do not, however, expect ever-receipt of citalopram while taking tamoxifen to be related to *CYP2D6* genotype, as this genotype would be unknown to the patient and provider at the first citalopram prescription. This study's results therefore pertain to the usual clinical setting. In addition, *CYP2D6* genotype is unlikely to cause citalopram prescription, or to share a common causal ancestor, so *CYP2D6* genotype does not satisfy the requisite causal structure of a confounder (Greenland *et al*, 1999). It may be possible that *CYP2D6* genotype is related to adherence to citalopram prescription or to long-term maintenance of the prescription, resulting from differences in the occurrence of adverse drug reactions in women with the different alleles. Such a relation could confound the association between breast cancer recurrence and duration of citalopram prescription while taking tamoxifen. Some non-randomized studies suggest such a relation between genotype and SSRI adherence (Rau *et al*, 2004; Zourková *et al*, 2007), whereas others suggest no such relation (Stedman *et al*, 2002; Gerstenberg *et al*, 2003; Roberts *et al*, 2004; Hedenmalm *et al*, 2006; Sugai *et al*, 2006; Suzuki *et al*, 2006). In the only randomized trial, *CYP2D6* genotype was not related to either the occurrence of adverse events or to adherence to paroxetine prescription (Murphy *et al*, 2003). Paroxetine is the most potent *CYP2D6* inhibitor of tamoxifen metabolism among the SSRI class (Jin *et al*, 2005). If *CYP2D6* genotype does not affect receipt or adherence to SSRI prescription, then it cannot confound the association we have reported.

Last, we do not know the indications for which citalopram was prescribed to the study participants, although ordinarily it would be prescribed primarily to treat depression. SSRI may also be prescribed to treat hot flushes (Stearns, 2006), but such prescriptions are rare in Danish breast cancer patients.

Weighing against these limitations are the strengths of the data quality. This study relied upon the Danish Breast Cancer Cooperative Group's registry of breast cancer patients, which provides clinical trial quality data in a population-wide setting in the four Northern Danish Counties. For example, the positive predictive value of breast cancer recurrence recorded by the DBCG equaled 99.4% in a validation study (Hansen *et al*, 1997), showing that there are few false-positive recurrences registered in the DBCG. In addition, of 1888 local and distant recurrences identified by medical record review among 4455 breast cancer patients assigned to a DBCG protocol, 1813 (96%) were correctly registered as recurrences in the DBCG database, 74 (3.9%) were identified as breast cancer deaths, and only 1 (0.05%) was not identified as either a recurrence or breast cancer death.

The prescription databases are generated by a computerised pharmacy accounting system that sends data to the Danish National Health Service, which refunds part of the costs associated with prescribed drugs. Given the direct connection between receipt of prescription medications and the pharmacy accounting system of the Danish National Health Service, we expect the prescription records to have excellent validity. The prescriptions from the four counties are merged into a research database at Aarhus University. In Denmark, antidepressants are available only at pharmacies and the patient must have a prescription from a medical doctor. Therefore, the county prescription databases are expected to have high sensitivity and specificity for ascertainment of citalopram prescriptions in the source population. Furthermore, because the prescription records antecede the date of breast cancer recurrence, they are a prospective data source presumably immune to differential classification bias (Rothman *et al*, 2008).

Despite these advantages, the study yielded only 17 cases of breast cancer recurrence among tamoxifen-treated women who had used citalopram while taking tamoxifen. The study was designed with 80% power to detect an OR of 1.6, and ultimately had 90% power to detect an OR of 2.3.

The results presented herein are, nonetheless, important and timely. A United States Food and Drug Administration advisory committee recently recommended relabelling tamoxifen with information on gene–drug and drug–drug interactions mediated by *CYP2D6* (American Cancer Society, 2007). Furthermore, the current practice guidelines of the United States National Comprehensive Cancer Network note that some SSRI reduce the formation of active tamoxifen metabolites, that citalopram and venlaflaxine appear to have minimal impact on tamoxifen metabolism, and that 'the clinical impact of these observations is not known' (National Comprehensive Cancer Network, 2008). Breast cancer patients taking tamoxifen and their physicians may therefore be concerned about SSRI comedication, even when antidepressants are strongly indicated. Our results suggest that citalopram prescription does not reduce tamoxifen's prevention of breast cancer recurrence.

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CORRESPONDENCE

Modification of Tamoxifen Response: What Have We Learned?

TO THE EDITOR: In a recent *Journal of Clinical Oncology* editorial, Desta and Flockhart[1] asked a yes-no question about the germline pharmacogenetics of tamoxifen response: "Have we learned enough?" They ultimately answer by writing that recommendations regarding genetic testing for *CYP2D6* variants must await further data, but the list of open questions preceding this answer did not include the question of whether there is a main effect. We therefore raise this question about the main effect: "Has it been established that tamoxifen is a less effective adjuvant therapy for estrogen receptor–positive breast cancer patients with functionally variant *CYP2D6* allele(s) than for those without functionally variant alleles?"

Figure 1 depicts the results of the five epidemiologic studies of this question.[2-6] To examine the departure of the distribution of these results from the null (ie, relative risk of 1.0), we first ranked the five relative risks of recurrence from lowest to highest (citations 2 to 6 in ascending order). We then plotted each study's relative risk and its 95% CI against the inverse normal of its rank percentile.[7] The stippled line shows the regression of inverse-variance weighted log-relative risk against the inverse normal of rank percentile. If the accumulated evidence is a random sample from a log normal distribution of relative risks centered on the null, then the relative risks should fall along this line and the line should intersect the *x*-axis at zero. The results of the accumulated studies fit just such a pattern. Indeed, the inverse-variance weighted geometric mean of the relative risks equals 1.04 (95% CI, 0.77, 1.41). Each study has certain strengths and limitations, some of which were pointed out by Desta and Flockhart, and consideration of them against one another is of value. Nonetheless, the simplest explanation for the results to date is that they are sampled from an underlying null association.

The integer above each interval in Fig 1 shows the number of women in the study (as best we can determine) with a recurrence and with the variant allele(s). Because both recurrence and the variant allele are rare, this number is the primary determinant of the study's precision. Summed over all of the studies, there are only approximately sixty women with both a recurrence and the variant allele(s), about half from studies reporting a protective association and half from studies reporting a causal association. In our view, sixty women, evenly distributed about the null, is too small a number to consider the association between *CYP2D6*-variant alleles and tamoxifen effectiveness to be precisely established.

Whether tamoxifen's effectiveness in the adjuvant setting is modified by gene variants and other medications is an important question. Based on the underlying pharmacology, ably reviewed by Desta and Flockhart in their editorial and elsewhere, it is reasonable to hypothesize that tamoxifen's effectiveness would be reduced by genetic variants or other medications that interfere with the metabolism of tamoxifen to its pharmacologically most active forms. However, the epidemiologic evidence accumulated to date does not support that conclusion. We therefore answer the question we posed at the outset with an unequivocal "no, it has not been established that tamoxifen is a less effective adjuvant therapy for estrogen receptor–positive breast cancer patients with functionally variant *CYP2D6* allele(s) than for those without functionally variant alleles." We encourage researchers to continue to generate and publish evidence that informs the answer regardless of whether the result comports with the compelling hypothesis suggested by the pharmacology.

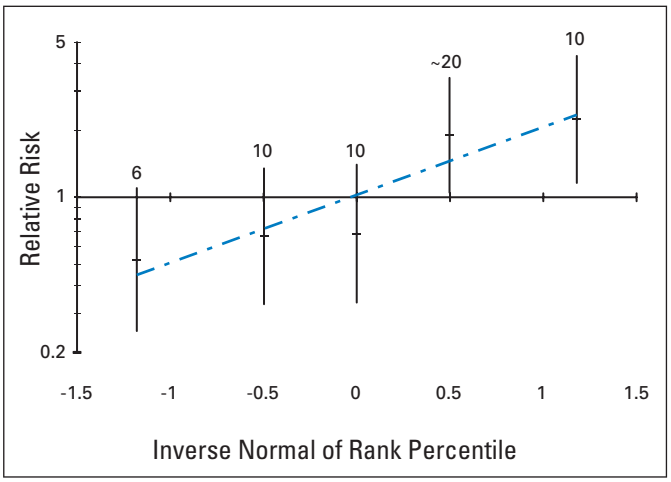

Fig 1. Relative risks and 95% CI from five epidemiologic studies of the association between *CYP2D6* variant allele and breast cancer recurrence (citations 2 to 6 in ascending order) plotted against the inverse normal or rank percentile. The stippled line shows the regression of inverse-variance weighted log-relative risk against the inverse normal of rank percentile.

Timothy L. Lash and Thomas P. Ahern
Department of Epidemiology, Boston University School of Public Health, Boston, MA

Deirdre Cronin-Fenton
Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

Jens Peter Garne
Department of Surgery, Aalborg Hospital, Aalborg, Denmark

Stephen Hamilton-Dutoit
Institute of Pathology, Aarhus University Hospital, Aarhus, Denmark

Marianne Ewertz Kvistgaard
Department of Oncology, Aalborg Hospital, Aalborg, Denmark

Carol L. Rosenberg and Rebecca A. Silliman
Department of Medicine, Boston University School of Medicine, Boston, MA

Henrik Toft Sørensen
Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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In Reply: We read with interest the comments of Lash et al,[1] on our recent editorial[2] describing the current status of germline pharmacogenetics of tamoxifen. Our piece was prompted by the article by Schroth et al[3] suggesting that patients who inherit nonfunctional alleles of cytochrome *P450 2D6* respond less well to tamoxifen treatment. We would like to thank these authors for giving us the opportunity to expand on the data underlying our opinion on the subject.

Our editorial attempted to address a series of outstanding questions that remain in this area, and to highlight the fact that much work remains. Lash et al[1] believe that we should have addressed more directly the key question posed by the data of Schroth et al[3]: whether tamoxifen response is influenced by *CYP2D6* variants at all. To support their argument, they present an analysis of data from five diverse studies, using the departure of the distribution of the relative risks from the relative risk of 1 (null). Each study's plot of relative risk (and 95% CI) was plotted against the inverse normal of its rank percentile. If all the studies were considered to be of equal size and quality, and no weighting was applied, this analysis showed that the relative risks fall in the regression line which intersects the *x*-axis at zero. The authors concluded that the accumulated evidence is a random sample from a log-normal distribution of relative risks centered on the null. On the basis of this line of evidence they contend that there is no evidence that tamoxifen is inferior in patients who carry *CYP2D6* variants associated with reduced or absent function.

We believe that the approach employed by these authors is based on the flawed assumption that it is appropriate to treat data obtained in carefully controlled, randomized, prospective trials (ie, level one evidence) with data obtained by analyzing samples in databanks where there was no prospective plan to collect any of the data analyzed, and where critical confounders such as drug dose, compliance, co-medication data, and stage of disease could not be adjusted for. Simply stated, the possibility of such potentially dangerous confounding is why we regard prospective and randomized trials as the gold standard of evidence. Only one of the studies[4,5] used by Lash et al in their analysis is such a prospective trial. The others are all vulnerable to various degrees of confounding.

In the analysis by Lash et al,[1] the relative risks from multiple reported results are ranked from low to high and they are close to a normal distribution. This is equivalent to averaging all five relative risks. Although such an approach might be useful in studies that are equivalent to each other, this simple approach is not appropriate to summarize data from multiple studies, especially retrospective nonrandomized studies in which patient and breast cancer heterogeneity can be great.

We would contend that the strength of evidence from retrospective studies cannot be equated with that obtained from prospective randomized trials. For example, Goetz et al,[4,5] reported that patients homozygous for the *4 genotype (*4/*4) or taking *CYP2D6* inhibitors had a worse recurrence-free time and disease-free survival than those with *1/4 and *1/*1 genotypes or those taking no inhibitors, showing the largest effects among those analyzed by Lash et al.[1] These data are derived from a prospective clinical trial, with a well-defined population of postmenopausal women and substantial (more than 12 years) follow-up, and, therefore, the strength of association and the size of effect seems more compelling in this study than most of the other studies that are retrospective in nature and involve less-characterized patients with breast cancer.

In addition, we believe that these authors omitted a number of recent studies showing associations between *CYP2D6* variation and reduced tamoxifen response in a range of settings including metastatic breast cancer,[6] breast cancer prevention,[7,8] and adjuvant therapy.[9] Of note, data from a second prospective randomized trial have been presented from the Italian tamoxifen prevention trial.[7,8] Data from these trials are consistent with those from the first prospective, randomized trial presented by Goetz et al,[4,5] and indicate an increased risk of disease recurrence in poor metabolizers of *CYP2D6*. Consistent with an important relationship between disease-free survival and *CYP2D6* activity, this study also showed that patients who carried multiple copies of the *CYP2D6* gene, associated with increased *CYP2D6* activity, experienced improved disease-free survival ($P = .0008$).[8] The analysis performed by Lash et al[1] includes data presented by Wegman et al,[10,11] who reported the opposite effect to those trials mentioned above,[3-9] and Nowell et al,[12] who reported no effect of *CYP2D6* variant on tamoxifen response. The reason for this discrepancy is not precisely known, but a higher drop-out rate from tamoxifen in extensive metabolizers who experience more severe hot flashes, as demonstrated recently by Rae et al,[13] is one potential explanation. In this study no poor metabolizers dropped out of a 297-patient trial with an overall 30% drop-out rate, suggesting that patients who stand to benefit the least stay on tamoxifen the longest, whereas those destined to derive the most benefit also experience the most adverse effects, and drop out most frequently.

Although we agree with Lash et al[1] that much more data are required and that many important questions remain, the key principles of the primacy of randomized, prospectively controlled trial data, and the importance of biologic and mechanistic plausibility should be taken into account in making key therapeutic decisions such as this.

Letter to the editor

Comment on 'Impact of *CYP2D6*10* on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy'

Timothy L. Lash,[1,2,3,6] Thomas P. Ahern,[1] Deirdre Cronin-Fenton,[2] Jens Peter Garne,[4] Stephen Hamilton-Dutoit[5] and Henrik Toft Sørensen[1,2]

[1]Department of Epidemiology, Boston University School of Public Health, US; [2]Department of Clinical Epidemiology, Aarhus University Hospital, Denmark; [3]Department of Medicine, Boston University School of Medicine, US; [4]Department of Surgery, Aalborg Hospital, Aarhus University Hospital, Denmark; [5]Institute of Pathology, Aarhus University Hospital, Denmark

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(*Cancer Sci* 2008; 99: 1706–1707)

Kiyotani *et al*. recently reported a study of the impact of the *CYP2D6*10* allele on recurrence-free survival in Japanese breast cancer patients receiving adjuvant tamoxifen therapy.(1) The **10* allele causes an amino-acid substitution that reduces the enzyme's functionality, so breast cancer patients with this allele who are treated with tamoxifen may not produce a sufficient concentration of the pharmacologically most active metabolites.(2) Indeed, compared with women who have the *1/*1 genotype, the authors reported a 4-fold higher rate of recurrence [95% confidence interval (95% CI) 0.41, 39.18] in women who have the *1/*10 genotype and a 16.63-fold higher rate of recurrence (95% CI 1.75, 158.12) in women who have the *10/*10 genotype.

The study included 67 women (a) diagnosed with estrogen or progesterone receptor-positive invasive breast cancer after 1985 at the Tokushima Breast Care Clinic, (b) treated with five years of tamoxifen and (c) agreeing to participate and give blood for genotyping in 2007. The authors acknowledged that patients with recurrences soon after diagnosis may not have participated because they were too ill or dead by 2007. The authors may not, however, have adequately considered the potential implications of studying recurrence rates in a time period that participants must have survived to join the cohort. All participants had to survive from their breast cancer surgery until their blood draw, yet recurrences and person-time in that period were included in the analysis. Outcomes and person-time that occur before the last event required for participation in a study are ordinarily excluded from analysis to avoid bias.(3) This study's cross-sectional design most likely introduced a selection bias, which can create the appearance of an association when none exists.(4)

For example, it is possible that the **10* allele actually delayed onset of recurrence or improved survival of patients who had a recurrence. Three of five earlier studies of the effect of the functionally variant *CYP2D6 *4* allele reported a lower recurrence risk in women with the variant allele.(5) The surviving breast cancer patients in 2007 would then have a higher prevalence of the **10* allele among patients with a recurrence – exactly as observed – but this higher prevalence would result from protection against recurrence and mortality by the **10* allele beginning at the time of diagnosis, not from a higher rate of adverse outcomes as suggested by the reported associations. Because of the study's susceptibility to bias, one cannot unambiguously interpret its result.

Nonetheless, if the study's results were approximately accurate, then the reported substantial increase in recurrence rate, in combination with the high *CYP2D6 *10* allele prevalence in Asian populations (approximately 18% **10/*10* homozygotes and 51% **10* heterozygotes),(6) should result in measurably poorer tamoxifen effectiveness in Asian populations than in Caucasian populations (in which the prevalence of *CYP2D6 *4/*4* homozygotes is only 5% and the prevalence of **4* heterozygotes is 27%).(6) As described below, surveillance and trial results supply no evidence to suggest that tamoxifen is less effective in estrogen receptor-positive Asian breast cancer patients than in estrogen-receptor positive breast cancer patients of other ethnicities. We recognize that these population-based comparisons cannot account for all of the potential differences in prognostic factors that affect the risk of breast cancer recurrence. Therefore these comparisons cannot inform definitive conclusions about the relative effectiveness of tamoxifen between Asian and Caucasian women.

In United States surveillance data, post-menopausal Asian/Pacific Island women had a breast cancer mortality rate 0.63-fold lower than white women (95% CI 0.48, 0.82).(7) The prevalence of estrogen receptor-positive tumors in the Asian/Pacific Island women (59.5%) approximately equaled the prevalence in white women (58.4%). In our study of post-menopausal breast cancer patients,(8) the proportion of Asian/Pacific Island women with estrogen-receptor positive tumors who received tamoxifen therapy (74%, unpublished datum) slightly exceeded the proportion in non-Hispanic white women (69%, unpublished datum). The **10* allele frequency is similar in native Japanese, 1st generation Japanese in the US, 3rd generation Japanese in the US, Koreans and Chinese,(6) so these observations are unlikely to be influenced by combining all Asian–American women into one group. Taken together, these data are inconsistent with the hypothesis that tamoxifen is less effective in Asian–American breast cancer patients than in European–American breast cancer patients.

More importantly, the risks of recurrence at five and 10 years after diagnosis in a Japanese tamoxifen trial are about the same as the risks of recurrence in primarily Caucasian women enrolled in tamoxifen trials. Post-menopausal breast cancer patients treated at the Cancer Institute Hospital in Tokyo between May 1989 and May 1995 (55% node-negative) received 2 years of tamoxifen therapy and were then randomized to receive three more years of tamoxifen therapy or to receive no further adjuvant endocrine therapy.(9) The data in Table 1 of the publication,

[6]To whom correspondence should be addressed. E-mail: tlash@bu.edu

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combined with the survival curves in Fig. 1 of the publication, allow calculation of the 5-year recurrence risk (12%) and the 10-year recurrence risk (17%) in women receiving about 5 years of tamoxifen. Among women with estrogen receptor-positive tumors randomized to receive about 5 years of tamoxifen (74% node-negative), the 5-year recurrence risk reported by the most recent overview of trials, most of which were conducted in predominantly Caucasian populations, equaled 15.1% and the 10-year recurrence risk equaled 24.7%.[10] These data are also inconsistent with the hypothesis that tamoxifen is less effective in Asian women.

The extent to which variant alleles of *CYP2D6* modify the effectiveness of tamoxifen therapy remains an open clinical question.[4] It is important to investigate the potential impact of alleles that are prevalent in populations of different ethnic descent, both to contribute to the evidence regarding the central hypothesis and to characterize any allele-specific differences in the gene–drug interaction. This study is difficult to apply towards those goals, however, because of its susceptibility to selection bias and because of its implausible effect estimates.

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Thomas P. Ahern, MPH
Boston University School of Public Health
Department of Epidemiology
715 Albany Street, T3E
Boston, MA 02118
Phone: (617) 638-5232
Email: tpa@bu.edu

Education:

2006: M.P.H., Concentration in Epidemiology, Boston University School of Public Health

1999: B.S., Biochemistry, University of Vermont

Professional Experience:

CURRENT POSITION:

D.Sc. Candidate, Epidemiology, Boston University School of Public Health, Boston, MA

RESEARCH:

2004-2006: Development Specialist, Shire Human Genetic Therapies (formerly known as Transkaryotic Therapies), Cambridge, MA.

2002-2004: Research Associate, Syntonix Pharmaceuticals, Waltham, MA

2000-2002: Laboratory Technician, Vermont Cancer Center, Burlington, VT

TEACHING:

2008: Teaching Assistant, Boston University School of Public Health, Epidemiologic Methods (EP712), Department of Epidemiology

2007-2008: Teaching Assistant, Boston University School of Public Health, Infectious Disease Epidemiology (EP755), Department of Epidemiology.

2006-2007: Teaching Assistant, Boston University School of Public Health, Modern Epidemiology (EP854), Department of Epidemiology.

1998-1999: Laboratory Teaching Assistant, University of Vermont, Biochemistry, Department of Agricultural Biochemistry.

HEALTH CARE:

1995-2002: Emergency Medical Technician, University of Vermont Rescue Squad, Burlington, VT

2001-2002: Poison Control Specialist, Vermont Poison Center, Burlington, VT

Honors:

1999: Walter Daniel Manz Leadership Award, Presented by the University of Vermont Rescue Squad, Burlington, VT.

Academics:

PRESENTATIONS:

March, 2009. Digoxin treatment is associated with an increased risk of breast cancer. Poster presentation. Boston University Science and Engineering Research Symposium. Boston, MA.

September, 2008. Cardiac glycosides and breast cancer. Departmental seminar. Boston University School of Public Health, Department of Epidemiology. Boston, MA.

March, 2008. Impact of acquired comorbidities on all-cause mortality rates among older women with breast cancer. Poster presentation. Boston University Science and Engineering Research Symposium. Boston, MA.

December, 2007. Selective serotonin reuptake inhibitors and modification of tamoxifen effectiveness in breast cancer patients. Poster presentation. San Antonio Breast Cancer Symposium. San Antonio, TX.

GUEST LECTURES:

March, 2009. Case-control studies. Guest lecture to Introduction to Epidemiology (EP711), Boston University School of Public Health.

February, 2009. Intervention studies. Guest lecture to Supplement to Introductory Epidemiology (EP911), Boston University School of Public Health.

October, 2008. Case-control studies. Guest lecture to Introduction to Epidemiology (EP711), Boston University School of Public Health.

GRANT SUBMISSIONS:

Thomas Ahern as Principal Investigator:

May 2007. Pre-doctoral fellowship for breast cancer research: Modification of tamoxifen effectiveness by gene polymorphisms, regulatory proteins, and other drugs. Proposal submitted to Department of Defense. PI: Ahern. 100% May 2008-May 2011. Funded April 2008.

May 2006. Pre-doctoral fellowship for breast cancer research: Modification of tamoxifen effectiveness by gene polymorphisms, regulatory proteins, and other drugs. Proposal submitted to Department of Defense. PI: Ahern. 100% September 2007-September 2010. Not funded August 2006.

MEMBERSHIPS:

2006-Present: Society for Epidemiologic Research. Student Member.

PEER REVIEWS:

2008: *European Journal of Epidemiology*
Preventive Medicine

2007: *Canadian Medical Association Journal*
Journal of Clinical Epidemiology

Publications:

PUBLISHED IN PEER-REVIEWED JOURNALS:

Ahern TP, Lash TL, Thwin SS, Silliman RA. Impact of acquired comorbidities on all-cause mortality rates among older breast cancer survivors. *Med Care.* 2009;47(1):73-9.

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